University of Alberta

THE EFFECT OF THE GLYCEMIC INDEX AND LOAD ON THE GLUCOSE AND GLUCO-REGULATORY HORMONAL RESPONSE AT REST AND DURING EXERCISE.

By

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ABSTRACT

The purpose of this thesis was to examine the effects of the glycemic index (GI) and glycemic load (GL) on glucose and gluco-regulatory hormones at rest and during exercise. Study one examined responses at rest. Twelve subjects consumed nutrition bars of low glycemic index/ low glycemic load (LGI/ LGL), high glycemic index/ moderate glycemic load (HGI/ MGL) and moderate glycemic index/ high glycemic load (MGI/ HGL). Study two examined responses during exercise. Five subjects consumed nutrition bars of LGI/ LGL, HGI/ MGL and MGI/ HGL 60 minutes prior to prolonged cycling. Ingestion of the LGI/ LGL bar resulted in augmented non-esterified fatty acids (NEFA) availability and hyperglucagonemia ($p < 0.05$) in both studies and attenuated insulin response and insulin/ glucagon ratio ($p < 0.05$) at rest versus the MGI/ HGL meal. All bars resulted in transient hypoglycemia before exercise ($p < 0.05$). At rest, the GI and GL ($r = 0.53$, $r = 0.54$ respectively, $p < 0.01$) equally predicted insulin responses. In summary, compared to HGL foods LGI/ LGL feedings evoke a lower insulin response but elevated glucagon and NEFA concentrations at rest and when consumed the hour prior to exercise.

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NOMENCLATURE

AA	Amino acids
ADP	Adenosine-Di-Phosphate
AMP	Adenosine-Mono-Phosphate
ANS	Autonomic nervous system
ATP	Adenosine-Tri-Phosphate
BCAA	Branch chained amino-acids
CHO	Carbohydrate
CNS	Central nervous system
E	Epinephrine
EGP	Endogenous glucose production
FFA	Free Fatty Acids
FOG	Fast twitch oxidative glycolytic
GH	Growth Hormone
GI	Glycemic Index
GIP	Gastric inhibitory polypeptide
GL	Glycemic Load
GP	glycogen phosphorylase
GS	glycogen synthase
HGI	High Glycemic Index
HGL	High Glycemic Load
HGP	Hepatic Glucose Production

HPAA	Hypothalamic pituitary adrenal axis
iAUC	Incremental area under the curve
tAUC	Total area under the curve
IMTG	Intramuscular Triglyceride
LGI	Low Glycemic Index
LGL	Low Glycemic Load
MGI	Moderate Glycemic Index
MGL	Moderate Glycemic Load
NE	Norepinephrine
NSP	Non-starch polysaccharides
PDH	Pyruvate dehydrogenase
PEPCK	Phosphoenolpyruvate carboxykinase
PFK	Phosphofrucktokinase
PK	Pyruvate kinase
RER	Respiratory Exchange Ratio
SA	Sympatho-adrenal
SD	Standard deviation
SO	Slow twitch oxidative
TCA	Tricarboxylic acid cycle
VLDL	Very Low Density Lipoproteins
VO_2	Oxygen consumption
$VO_{2\,max}$	Maximal oxygen consumption

CHAPTER 1 - INTRODUCTION

The glycemic index (GI) is a standardized system of classification for carbohydrate (CHO) foods based on their postprandial blood glucose response. The calculation for the GI equals incremental area under glycemic response curve of 50 grams test food/ incremental area under glycemic response curve of 50 grams reference food*100 [1]. The GI is considered a measure of CHO quality and relates to the rate at which a food is digested and absorbed [2,3]. Given that CHO food intake in a diet typically exceeds 50 grams the GI has been criticized for inadequately characterizing the glycemic response to portion sizes exceeding this amount or whole diets [4]. Since the glycemic effect of a meal or diet is a product of both the CHO quality and quantity, the glycemic load (GL) was introduced in an attempt to more accurately quantify the overall glycemic response [5,6]. The calculation for the GL equals (GI*Grams of available CHO)/ 100. Proponents of the GI and GL advocate their efficacy in the prevention and management of chronic conditions through the prescription of a varied low glycemic diet [7-10]. Debate persists however regarding their clinical utility [2,11-16].

Studies that have observed the acute resting metabolic events to consuming foods of differing GI, have consistently demonstrated greater glycemic and insulinemic responses and lower free fatty acid (FFA) concentrations following high glycemic index (HGI) compared to low glycemic index (LGI) meals in healthy [17-20] but not always diabetic individuals [15,21]. Few studies have determined the acute counter-regulatory hormonal responses based on the GI or GL, which is necessary to fully characterize the postprandial gluco-regulatory stress to these indices. Observations from obese and healthy patients suggest that HGI meals elicit augmented glycemic and insulinemic

responses but suppress glucagonemic, FFA and glycerol levels compared to LGI meals during the early postprandial period [22-24]. Evidence also suggests that the GL may be a better predictor of the insulinemic effect of a meal in healthy individuals [25]. Few, if any studies have investigated the effects of both the GI and GL on metabolic and gluco-regulatory hormonal responses to meals in healthy individuals.

Carbohydrate feedings before and during prolonged exercise are essential for optimal endurance performance [26-28]. A potential disadvantage of CHO intake before exercise is a transient fall in blood glucose at the start of exercise due a greater rate of peripheral glucose extraction relative to hepatic glucose production (HGP) [29]. The efficacy of consuming CHO the hour prior to exercise is also controversial due to evidence suggesting that this nutritional strategy may increase [30,31], have no effect [32-34], or decrease [35,36] endurance performance. The GI has been advocated as a useful method for manipulating CHO intake in sports nutrition [37]. When CHO consumption the hour before exercise is quantified, the GI literature shows that LGI foods elicit smaller postprandial glycemic and insulinemic perturbations compared to HGI foods [33,38,39] and may [30,31,40] or may not improve performance [33,34,41]. Some authors have advocated the consumption of LGI foods the hour prior to exercise [37], but evidence appears to suggest a lower bioavailability during exercise compared to HGI feedings [42,43]. To the best of the authors' knowledge no studies have examined the metabolic and gluco-regulatory hormonal responses to CHO foods consumed the hour before exercise. This would help to clarify the differential benefits of consuming foods based on the GI during this period.

Controversy exists regarding the GI's efficacy in predicting the glycemic response to mixed meals given that it only documents the blood glucose response to single foods.

However glucose response is based on a myriad of factors that affect the magnitude and duration of postprandial glycemia [44-51], including the co-ingestion of fat and protein with CHO [45,47-49,51]. Some studies support the GI's predictive capabilities in this context [11,17,21,52], while others do not [12,13,53-55]. Nutrition bars are a convenient and readily accessible food-source consumed at rest and during exercise. Given their balanced macronutrient composition and known GI and GL values [3,56,57] they would appear to negate previous criticisms and facilitate valid investigation into the efficacy of these indices in predicting postprandial metabolic responses to meals containing significant quantities of CHO, protein and fat. It is difficult to find published research that has characterized the metabolic and gluco-regulatory hormonal responses to the GI and GL in nutrition bar form, either at rest or during exercise.

The first study of this thesis aimed to examine the effects of the GI and GL on the metabolic and gluco-regulatory hormonal responses at rest. Healthy males were asked to consume a LGI/ LGL, HGI/ MGL or MGI/ HGL meal. Postprandial glucose, NEFA, insulin and glucagon responses was determined over 150 minutes. It was hypothesized that: (i) ingestion of LGI/LGL compared to HGI/HGL nutrition would elicit an attenuated glycemic and insulin/ glucagon ratio response; and, (ii) the GL will better correspond to the insulinogenic effects of the meals.

The second study examined the effect of the GI and GL on the glucose and gluco-regulatory hormonal responses during prolonged cycling. Trained male cyclists were asked to exercise for 105 minutes, 60 minutes after the consumption of either a LGI/ LGL, MGI/ MGL or MGI/ HGL meal. Glucose, NEFA, insulin, glucagon and cortisol responses were determined. It was hypothesized that: (i) ingestion of LGI/ LGL

compared to HGI/ HGL nutrition 60 minutes prior to exercise would increase glucagon and cortisol concentrations during exercise; and (ii) ingestion of LGI/ LGL nutrition would negate the transient hypoglycaemic and hyperinsulinemic perturbations observed following HGI nutrition at exercise onset.

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CHAPTER 2 - REVIEW OF LITERATURE

2.1: Energy kinetics during prolonged moderate intensity endurance exercise

The increased metabolic requirements of skeletal muscle during exercise are met by the activation of the sympathoadrenal (SA) division of the autonomic nervous system (ANS) [1,2]. The SA systems associated messengers' epinephrine (E) and norepinephrine (NE) (catecholamines) partially control metabolism throughout the entire range of exercise intensities and demonstrate feed-forward characteristics allowing for a graded response proportional to exercise intensity and volume [3,4].

Moderate intensity exercise (50% to 75% of $VO_{2\ max}$) results in the recruitment of both slow twitch oxidative (SO) and fast twitch oxidative glycolytic (FOG) motor units associated with type I and IIA muscle fibers respectively, based on the relationship between motor unit size and excitability [5,6]. Innervation of type IIA muscle fibers along with augmented concentrations of circulating E and NE and their vasoconstrictive effects at the adipose tissue and splanchnic bed, results in an equal partitioning of energy provision between endogenous and peripheral energy stores at moderate intensities of exercise [7-9]. At the onset of exercise at an intensity of 65% of $VO_{2\ max}$ approximately 50% of energy is derived from carbohydrate (80% muscle glycogen and 20% blood glucose), while the remaining 50% of energy expended is derived from lipid including free fatty acids (FFA) from adipose tissue and to a lesser extent intra-muscular triglyceride (IMTG) [10-12].

At exercise intensities above 50% of $VO_{2\ max}$ plasma E and NE concentrations have been shown to increase four to six-fold [7,13]. Catecholamines directly stimulate lipolysis in the adipose tissue and the catabolism of IMTG stores [13,14]. Adrenergic

stimulation of lipolysis during exercise is also significantly aided by its inhibition of insulin secretion from β cells in the pancreatic islets [2,16]. Suppression of insulin release reduces its anabolic actions such as proteogenesis, lipogenesis and glycogenesis along with its antilipolytic and hypoglycemia effects including inhibition of lipolysis, glycogenolysis, gluconeogenesis [17].

Muscle contraction along with increased concentrations of circulating E result in the activation of glycogen phosphorylase (GP) and subsequent augmentation of skeletal muscle glycogenolysis [18]. Glucose produced is metabolized within the exercising musculature and not released into the circulation [19]. In addition to insulin-stimulated glucose uptake skeletal muscle contraction mediates the augmentation of peripheral glucose extraction via a contraction-induced pathway, which also facilitates the translocation of GLUT-4 transporters from dedicated intracellular pools to the muscle sarcolemma [20,21]. As muscle glycogen is degraded during prolonged exercise energy is derived increasingly from blood borne glucose [7,11].

Augmentation of blood glucose uptake at the muscle during prolonged endurance exercise is replenished by hepatic glucose production (HGP), initially via hepatic glycogenolysis, followed by hepatic gluconeogenic production of glucose from lactate, pyruvate, amino acids (AA) and glycerol once liver glycogen stores have been reduced [22,23]. Initial release of glucagon from α cells in the pancreatic islets is stimulated by transient hypoglycemia, a consequence of augmented peripheral glucose uptake [24]. Glucagon acts exclusively at the liver facilitating hepatic glycogenolysis at lower and gluconeogenesis at higher plasma glucose concentrations as hepatic glycogen is depleted [24-26]. Aided by the ancillary actions of the catecholamines, glucagon stimulates

gluconeogenesis through the activation of the gluconeogenic Cori and glucose-alanine cycle's [27-29], while it also indirectly stimulates lipolysis [30]. As exercise volume increases concurrent elevations in concentrations of NE and E stimulate glucagon secretion in a feed-forward fashion [31].

Exercise, along with centrally detected reductions in blood glucose via the gluco-regulatory hypothalamic center activates the hypothalamic-pituitary-adrenal axis (HPAA) resulting in secretion of cortisol from the adrenal cortex [19]. Increased concentrations of cortisol stimulate protein degradation to a greater extent than proteogenesis, whilst also indirectly enhancing lipolysis [32-34]. Resultant elevations in the concentration of circulating alanine and other AA concentrations serve as a source for hepatic gluconeogenesis [29,35,36]. Increasing concentrations of cortisol and E with increasing exercise volume also amplify each other's secretion in a feed-forward fashion [3].

High concentrations of catecholamines and other counter-regulatory hormones such as glucagon, cortisol and growth hormone (GH) and adrenocorticotropic hormone (ACTH) also increase the supply of gluconeogenic precursors glycerol, lactate and alanine to the splanchnic bed [10-12] (Figure 2.1). The lipolytic actions of the gluco-regulatory hormones in concert with their glucose sparing effects also mediate the progressive shift toward augmented fat oxidation reported with prolonged exercise, as their own concentrations rise [7].

2.2: Muscular fatigue and prolonged endurance exercise

Muscular fatigue has been defined as the failure to maintain a given or desired power output leading to reduction in performance [37]. For exercise intensities

synonymous with prolonged endurance activity (65-85% of Vo_{2max}) evidence suggests that muscle glycogen depletion and hypoglycemia appear to be important contributing factors to fatigue [38-41]. Hypoglycemia observed during prolonged exercise is thought to be the result of an imbalance between hepatic gluconeogenic glucose output and peripheral blood glucose extraction by the working musculature [10,11,37,41]. This has led to the conclusion that CHO oxidation is essential for the maintenance of prolonged endurance exercise at high intensities (65-90% $Vo_{2\ max}$) via its supply of critical metabolic intermediates, such that CHO depletion limits the oxidation of fat and protein [37]. Newsholme & Leech postulate that a loss of muscle glycogen results in an inability to sustain glycolytic flux rate and adenosine-tri-phosphate (ATP) regeneration [42]. Empirical evidence has also documented a correlation between muscle glycogen depletion and the accumulation of by-products of ATP hydrolysis that are indicative of impaired ATP resynthesis [43,44]. Sahlin et al elaborates further suggesting that the reduction in ATP resynthesis observed is a consequence of deficient concentrations of pyruvate, resulting from a reduction in CHO oxidation [45]. This appears to result in the deficient supply of intermediate substrates for the tricarboxylic acid (TCA) cycle necessary for sustained oxidation of FFA and AA. As a result the energy available for ATP resynthesis is reduced making it impossible for the muscles to sustain the necessary contractile function for maintenance of work output and endurance performance [45,46].

2.3: Evidence for the role of exogenous carbohydrate in delaying fatigue

Findings that the ingestion of exogenous CHO prior to or during prolonged exercise may improve endurance performance and delay the onset of fatigue underpin suggestions of a causative role for muscle glycogen depletion in the etiology of muscular

fatigue [47]. Carbohydrate supplementation (glucose or glucose polymer solutions) has been shown to improve the maintenance of euglycemia, augment CHO uptake and oxidation and attenuate lipolysis [48-52]. Most evidence investigating muscle glycogen use in response to CHO supplementation however suggests glycogenolysis is unaffected despite elevated glycemia and glucose uptake [49,52,53]. Hyperglycemia is thought to substitute for depleted muscle glycogen enabling an increased reliance on blood glucose as energy supply shifts from endogenous stores to circulating substrates with increasing duration of exercise [7,48-51]. Compared to exercising in the fasted state, CHO consumption results in hyperinsulinemia during exercise, which augments peripheral glucose uptake, glycolysis and CHO oxidation, whilst inhibiting lipolysis and HGP [29,48,49,54,55]. Mitchell et al. suggests that such a metabolic perturbation may spare hepatic glycogen stores [55]. Higher levels of CHO oxidation likely facilitate the maintenance of pyruvate production and the provision of obligatory TCA cycle intermediaries necessary for the oxidation of fat and AA, thus negating impaired ATP resynthesis often observed whilst exercising in the post-absorptive state [56].

2.4: Carbohydrate Assimilation

The jejunum and ileum represent the middle and distal divisions of the small intestine and serve as the primary absorptive surfaces of the gastrointestinal tract. Mucosal enzymes, particularly disaccharidases and peptidases complete the digestive processes initiated by pancreatic enzymes in the lumen [57,58]. Partially digested CHO is broken down via the actions of disaccharidases (maltase, isomaltase, lactase, sucrase, trehalase) located on the luminal surface of the enterocyte into monosaccharides [57-59].

Sucrase splits sucrose, producing glucose and fructose, lactase breaks down lactose producing glucose and galactase, while trehalase breaks down trehalose into glucose [57]. Due to the large disaccharidase reserve in the small intestine, the rate-limiting step in CHO assimilation is not digestion but its absorption following hydryolsis [57]. The products of CHO digestion, glucose, fructose, galactose (monosaccharides) are then absorbed across enterocytes lining the intestinal epithelium and then they enter the vascular system. Fructose is absorbed by facilitated diffusion and cannot be energized for active transport. Glucose and galactose are absorbed by secondary active transport via a Na^+ dependent carrier system. Due to their dependence on a common carrier for intestinal absorption, an abundance of one can inhibit the transport of the other across the intestinal epithelium [57-59]. For one glucose molecule bound to a carrier two Na^+ are also transported. ATP drives the sodium pump and sustains a Na^+ gradient favoring glucose entry. The exit of glucose into the intracellular space is attributed to facilitated diffusion via a Na^+-independent carrier located at the basolateral membrane of the enterocyte [57-59].

2.4.1: Glucose assimilation: A small portion of the glucose absorbed by intestinal enterocytes is metabolized via glycolysis for ATP generation and appears as lactate in the hepatic portal vein [60]. Following secondary active transport across the intestinal enterocytes and out of the basolateral membrane however, the majority of exogenous glucose enters the portal vein and subsequently passes through the liver and eventually the systemic circulation [57-59]. Augmented concentrations of circulating glucose (> 80-85 mg/dl) serve as the primary stimulus for insulin release into the systemic circulation via diffusion into islet β cells [19,26]. Insulin's anabolic actions stimulate cellular uptake and

glucose disposal via insulin-sensitive tissues through the translocation of GLUT-4 transporters from a distinct intra-cellular pool to the plasma membrane [19]. Elevated concentrations of insulin suppress HGP by inactivating the glycogenolytic enzyme GP, inhibiting synthesis of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK), whilst also augmenting glycogen synthase (GS) activity [29,61]. Increased concentrations of insulin also direct cellular metabolism towards CHO oxidation by stimulating glycolysis, via activation of phosphofrucktokinase (PFK), pyruvate kinase (PK) and pyruvate dehydrogenase (PDH) [19].

Assimilation of exogenous glucose and the return of plasma glucose to basal levels is the result of reduced endogenous production along with accelerated glucose uptake by the liver, muscle and adipose tissue [61]. Woerle et al reported a 58% depression in HGP following the ingestion of a 78g oral glucose load. While endogenous glucose output still contributed 28% of glucose entering the systemic circulation postprandially, a significantly greater proportion of this was supplied via gluconeogenesis, indicating a relatively greater suppression of glycogenolysis as the primary mediator in attenuating HGP compared to the post-absorptive state [62]. During the postprandial period disposal of exogenous glucose by splanchnic tissues has been shown to account for approximately 30% of an ingested glucose load, the majority of which would be taken up by the liver [62]. Glycolysis is the predominant source of disposal for splanchnic tissues while the remaining glucose contributes towards hepatic glycogenesis [62]. Peterson et al reported that 17% of an orally ingested glucose load (98g) was disposed of as hepatic glycogen. Of this amount 63% of hepatic glycogen formation resulted from direct production from glucose, while 37% originated from indirect pathways, namely gluconeogenic production

of glucose originating from the oral glucose load [63]. The majority (~ 70%) of ingested glucose however enters the systemic circulation and is disposed of by non-hepatic tissue, namely muscle and adipose tissue with the predominance of this portion undergoing glycolysis (67%), while 32% (22% of total glucose load ingested) is directly stored as glycogen [63]. The observations of Taylor et al however demonstrated that between 26 and 35% of an orally ingested glucose load is disposed of as muscle glycogen indicating a higher proportion may be directly stored in the periphery [64].

2.4.2: Galactose assimilation: Following facilitated active transport across the intestinal enterocytes galactose enters the hepatic portal vein, is transported to the liver and into the systemic circulation. No catabolic pathways exist to metabolize galactose. Consequently it is converted into a metabolite of glucose, glucose-6-phosphate in a series of four steps enabling ingested galactose to eventually enter the metabolic mainstream [65]. The liver appears to be the primary organ involved in the metabolism of galactose being particularly well-endowed with the 4 enzymes that constitute the Leloir pathway. Clearance of galactose by the liver however has been shown to reach saturation at plasma levels of 50 mg/100ml [66]. The galactose metabolic pathway (uridine nucleotide or leloir pathway) involves the reversible inter-conversion of galactose and glucose via the actions of 4 specific enzymes, galactomutarotase, galactokinase, galactose-1-phosphate uridyltransferase and UDPgalactose-4-epimerase [66,67] (Figure 2.1).

Galactomutarotase initially epimerizes β-D-galactose to α-D-galactose. Galactokinase then catalyzes the ATP-dependent phosphorylation of α-D-galactose resulting in the production of galactose-1-phosphate (and ADP). Galactose-1-phosphate

uridyltransferase then adds a uridyl group (UMP) from uridine diphosphate glucose (UDP-glucose) to galactose-1-phosphate, producing UDP-galactose and glucose-1-phosphate. UDP-galactose-4-epimerase finally catalyzes the inversion of the hydroxyl group at carbon 4 on UDP-galactose, which is consequently epimerized to UDP-glucose [65-67]. UDP-glucose is an activated form of glucose, and an important donor in glycogenesis. A portion of ingested galactose is consequently converted into the inert storage form of glucose, glycogen via the actions of GS. Alternatively the enzyme UDP-glucose pyrophosphorylase catalyzes a final phosphotransferase reaction involving UDP-glucose and two phosphate molecules resulting in the formation of UTP and glucose-1-phosphate. Further conversion of glucose-1-phosphate into glucose-6-phosphate via the actions of phosphoglucomutase enables the end product to enter cellular metabolism as a glycolytic intermediary [65-67] (Figure 2.1). Little empirical evidence exists however quantifying the routes of galactose disposal by different tissues due to its role in synthetic reactions. The main method of galactose assimilation however is the uridine nucleotide pathway primarily at the liver although erythrocytes and the kidney play a significant role [66].

2.4.3: Fructose assimilation: Debate exists regarding the physiological effect of fructose. During the early digestive process evidence exists demonstrating that fructose is emptied from the gut at a much faster rate (exponentially) when compared to glucose (linear) [68]. Conversely, intestinal absorption of fructose across the mucosa appears to be rate limiting, occurring more slowly when compared to glucose absorption due to its dependence upon facilitated diffusion and not active co-transport [69,70]. Following

facilitated diffusion of fructose across the intestinal epithelium, fructose enters the hepatic portal vein and consequently passes through the liver. Due to the existence of an active hepatic enzyme system for the metabolism of fructose a significant portion (~ 50%) of that ingested is rapidly extracted by the liver following oral feeding. Nilsson & Hultman demonstrated that fructose can give rise to four times as much liver glycogen when compared to glucose infusion, underlying the integral role of the liver in fructose metabolism [71]. The remaining fructose in the systemic circulation is taken up by the kidneys (~ 20%) while smaller quantities are extracted by adipose tissue and skeletal muscle [72]. As a result ingestion of fructose results in lower total blood sugar concentrations when compared to glucose consumption [65]. Furthermore empirical evidence has demonstrated that compared to glucose, different doses of fructose appear to elicit no marked rise in serum insulin concentrations, suggesting cellular uptake of fructose occurs via insulin independent pathways [65].

With regard to hepatic (and kidney) fructose metabolism, specific enzymes (fructokinase, aldolase B and triokinase) result in its conversion into dihydroxyacetone-phosphate and glyceraldehydes-I-phosphate (both of which are glycolytic intermediaries), which belong to the triose phase of metabolism, by-passing the rate controlled step of glycolysis catalyzed by PFK (Figure 2.1). Fructose consequently provides increased substrate for glycolysis, gluconeogensis, glycogenesis, lipogenesis and fatty acid esterification [72]. The fate of ingested fructose however is contingent on the underlying metabolic circumstance. In the fed state, the majority of intermediaries produced from hepatic fructose metabolism saturate the glycolytic pathway, resulting in augmented lactate production and elevated concentrations of blood lactate following ingestion [72-74].

Glucose production (glycogenolysis, gluconeogenesis), glycogenesis and lipogenesis tend not to be enhanced. Increased concentrations of dihydroxyacetone and acetyl CoA (as a result of augmented glycolytic flux) also however elevate substrate supply for the esterification of long chain acetyl CoA, and the consequent synthesis of trigylceride and phospholipids. Increased triglyceride formation being the primary precursor and determinant of very low density lipoproteins (VLDL) also results in their synthesis and secretion from hepatocytes [72]. In the fasted state the underlying hormonal milieu along with reduced hepatic concentrations of fructose-2-6-biphosphate, results in the activation of fructose-1-6-biphosphatase and the gluconeogenic pathway, whilst inhibiting PFK and glycolysis. The result is an augmentation of fructose conversion into glucose [72].

2.5: Factors affecting the rate of digestion and absorption

A number of factors affect the magnitude and duration of the postprandial glycemic response following the ingestion of food. These include the type of carbohydrate consumed, the co-ingestion of other macronutrients, caloric content, meal volume, food preparation, and exercise, amongst others [75-86].

2.5.1: Type of CHO: An abundance of empirical evidence has documented the effect of CHO type on the postprandial glycemic and gluco-regulatory hormonal response. A large number of studies have demonstrated that some complex CHO produce similar and in some cases larger glycemic responses when compared to simple sugars [80]. A high amylopectin/ amylose ratio for example within a starch has been shown to lower the glycemic and gluco-regulatory responses. Such an observation has been attributed to

structural differences, with amylopectin, a branch chained starch having a larger surface area for amylytic attack (amylose digestion) compared to the straight-chained amylose [80,81]. CHO foods higher in soluble non-starch polysaccharides (NSP) (dietary fiber) have also been shown to elicit a significantly attenuated postprandial glucose and insulin response [82]. Mechanisms postulated to mediate such a response include a reduced mixing of gut contents with digestive enzymes and a mechanical effect of the viscous components of NSP's increasing propogation within the intestine and reducing contact of CHO with the digestive surfaces of the intestinal epithelium [81,82].

2.5.2: Co-ingestion of fat: Studies investigating the effect of the co-ingestion of fat along with CHO have demonstrated a lower glycemic response compared to CHO alone [76,79]. The empirical consensus from such studies support delayed gastric emptying as the primary reason for such observations. A large mass to surface area ratio of fat and carbohydrate complexes causing a reduction in CHO availability for amylytic attack has also been cited as another possible mediator [79]. With regard to the insulin response there is strong contention that co-ingestion of fat maintains normal postprandial insulin levels despite decreases in blood glucose, an observation which Collier et al attributes to an insulin de-sensitizing effect of fat [76]. Further evidence however suggests that not all types of fat have the same effects with n-3 fats thought to increase insulin secretion to a greater extent than n-6 in normal subjects [84].

2.5.3: Co-ingestion of protein: The co-ingestion of protein along with CHO has also been demonstrated to elicit a reduced postprandial glycemic response [85,86]. Contrary

to observations with fat however protein consumption has been shown to elicit an increased insulinemic response, due to the stimulatory effects of amino acids on pancreatic insulin secretion [85,87]. Andersen et al attributed the lower glycemic response observed following protein co-ingestion to a protein-CHO complex that reduces the rate of digestion and absorption [88]. Evidence by Nutall et al also suggests that the co-ingestion of protein with CHO may also have a stimulatory effect on pancreatic glucagon secretion. Such an observation was attributed to the dependency of circulating glucagon concentrations on the protein-CHO ratio of food [89].

2.5.4: Energy intake: The caloric content of food is considered the primary determinant of gastric emptying. Gastric motility is inversely related to the energy density of ingested food, while calorically inert solutions have been shown to leave the stomach faster than fluids containing calories [90,91]. Meals of the same volume and calorie content but consisting of different macronutrients have also been shown to be emptied from the stomach at similar rates [92,93]. Brener et al observed the gastric motility of different concentrations of CHO solutions and concluded that emptying slowed as the glucose concentration and subsequently the energy density of the solution increased [94]. Such observations have also been confirmed elsewhere [95,96].

2.5.5: Meal volume: Thomas outlines that for any given individual the amount of chyme evacuated in a given time from the stomach is a function of the volume of the gastric contents [97]. Hunt & MacDonald have previously outlined that the propulsive drive of the stomach is proportional to intra-gastric volume [98]. Mechanoreceptors in the

stomach musculature respond to increasing gastric distension and pressure by increasing the force of antral contractions and subsequent emptying into the duodenum. Following a large meal when gastric volume is greatest the force of peristaltic contractions are elevated, resulting in the increased ejection of chyme and the augmentation of gastric motility [60]. Consistent with such, Hunt and coworkers observed a significant increase in gastric emptying through doubling the volume of meals beyond what was observed through doubling the energy density of meals [99].

2.5.6: Food form/ preparation: While food arriving at the stomach is a mixture of solids and liquids, the passage of gastric contents through the pyloric sphincter as chyme into the duodenum is essentially a matter of liquid movement [97]. The anatomy of the stomach therefore means that ingested beverages will be emptied from the stomach at a faster rate than solid food forms due to an increased necessity for enzymatic digestion before the latter can pass through the pyloris and into the duodenum. Haber et al observed the effects of liquid, gel and solid foods on the blood glucose response to determine the differential rate of digestion and absorption for each [99]. Findings revealed that while all forms produced strikingly similar peak blood glucose concentrations, solids produced a far more prolonged elevation which is suggestive of either attenuated gastric emptying and/ or intestinal absorption [99].

A large portion of evidence has also investigated the differential effects of food processing on the rate of gastric emptying and absorption. Jenkins et al observed the traditionally slow augmentation of blood glucose following consumption of legumes to be highly heat labile [75]. Gatti et al also demonstrated the degree of cooking and heat

processing to account for the differential glycemic responses following the ingestion of identical types and quantities of boiled and boiled-baked rice. The authors postulated that the lower glycemic response following baking was attributable to major changes on the starch molecule, heavily reducing the water content of rice, resulting in a more compact granule [100]. Alternatively, starch granules have been shown to swell and rupture in moist heat (i.e. boiling) which may have made the granules more accessible to enzymatic digestion and explain the higher glucose and insulin response observed [100]. Vaaler and coworkers have also reported similar observations [101].

Further studies have also investigated the effect of physical form on the speed of carbohydrate absorption. Wong et al examined the effects of viscosity and physical form (using whole, blended and ground lentils) on the postprandial glycemic response. Observations revealed that while increased viscosity failed to affect the rate of glucose release, grinding lentils into fine particles resulted in significantly greater starch hydrolysis rates compared with whole grain lentils [102]. The authors postulated that the fibre in lentils reduces the rate of hydrolysis by forming a physical barrier that insulates the starch from hydrolytic enzymes, limiting their accessibility to the substrate. Grinding the food appears to remove the actions of fibre as a physical barrier in its natural form and increase the surface area/ starch ratio allowing the rate of starch hydrolysis to increase [102]. Other investigations have also reinforced such observations [81,103].

2.5.7: Exercise: Empirical evidence suggests that at moderate exercise intensities no significant alteration in gastric emptying or intestinal absorption of CHO occurs [55,104-106]. Some studies suggest that compared to rest gastric emptying may be augmented

during exercise, due to increased movement of fluid within the stomach, especially when running [107]. Cammack et al also reported the acceleration of gastric emptying following a solid pre-exercise meal while cycling, suggesting that such an effect may not be isolated to ballistic exercise modalities [55,108]. Contrastingly exercise intensities greater than 70-80% Vo_{2max} have been shown to inhibit gastric emptying. Increased sympathetic outflow and the resultant catecholamine release are thought to mediate such a response through the inhibition of gastrointestinal function/ motility and gut blood flow [55,106,109].

2.6: Glycemic Index (GI)

The glycemic index (GI) is a standardized system of classification for CHO containing foods based on their postprandial blood glucose response [87]. The GI is calculated as a percentage value based on the area under the blood glucose response curve to 50 grams of a CHO containing food, divided by the area of the blood glucose response curve of 50 grams of a CHO reference food, glucose or more typically, white bread [110].

$$\mathbf{GI} = \left(\frac{\textbf{Incremental blood glucose area of test food (2 hrs)}}{\textbf{Incremental blood glucose area of reference food (2 hrs)}} \right) \textbf{x 100}$$

Using the GI, foods can be pooled based on different glucose tolerances as having a low (< 55%), moderate (56-69%) or high (> 70%) glycemic response [110] (Tables 2.1-2.3). The GI response of foods relates to the rate at which they are digested and absorbed. Slowly digested foods elicit a flatter, more prolonged glycemic response while rapidly digested foods produce larger, transient excursions in blood glucose and more

pronounced physiological perturbations (Fig 2.2) [87,110]. Since its conception, the GI of a plethora of CHO containing foods has been determined [111].

The GI concept was originally constructed as an aid for diabetic populations in making appropriate dietary choices based on the inability of previous guidelines to accurately predict the postprandial blood glucose response to CHO containing foods [110,112-114]. Proponents of the GI concept have suggested its potential efficacy in the prevention and management of chronic conditions and diseases (specifically diabetes mellitus) through the prescription of a varied diet of low glycemic foods in order to maintain tighter glucose control [112].

2.6.1 GI controversies

(i) Factors affecting the rate of digestion and absorption: Opponents of the GI, cite the myriad of factors that affect a foods GI, with strikingly similar foods or even the same food eliciting vastly different postprandial glycemic responses as a consequence of differences in methods of food preparation, CHO load, caloric intake, meal volume, type of CHO, ripeness of food, and food form [55,80-86,95-109,113,115]. Proponents of the GI concept however argue that its' very strengths lie in its ability to document and list differences in the glycemic response in a standardized manner based on such sources of variation [87].

(ii) Consistency of predictive capabilities: Another matter of contention is the capability of the GI in consistently predicting the postprandial blood glucose response both between and within individuals. The GI was designed to eradicate inter-individual variation based on the use of relative individual responses to a standardized reference

food. However, large disparities in the GI between different individuals to the same food have been reported by some authors, questioning its efficacy in doing so [118,119]. Further observations also question the predictive capability of the GI in ranking the glycemic response of different foods within individuals (intra-individual variability). Coulston and co-workers [118] compared the GI of four common CHO containing foods and reported significant inconsistencies in the relative rank order of each within individuals, implying that the glycemic response may be idiosyncratic in different individuals. Wolever et al however, attributes such observations to day-to-day variability in the glycemic response within subjects, which were unaccounted for due to the limited number of occasions on which the glycemic response to each test food was determined in these investigations [120]. Consequent studies from Jenkins and co-authors during which the GI of each test food was used taken on multiple occasions appear to support the GI's predictive capability [120].

(iii) Mixed meals: The biggest area of consternation remains the GI's efficacy in predicting the postprandial glycemic response to mixed meals as the GI construct only documents the blood glucose response to single foods. Co-ingestion of protein and fat either with or within CHO containing foods has been shown to significantly alter the glycemic response [76,79,89,120]. Wolever & Jenkins worked to redress this issue by constructing a method for predicting a meal GI (MGI) based on both the amount of CHO provided by and the GI of each constituent food [121]. Firstly the total meal CHO in grams (g) is calculated by summing the CHO loads of each food ($g = g_a + g_b + g_c + g_d$ .....). The proportion of CHO provided by each food to the total meal CHO load is then calculated ($P_a = g_a/g$) [121]. This value is then multiplied by the GI for that food to provide a MGI of

that food to the total MGI ($MGI_a = P_a \times GI_a$). Finally the MGI's of each constituent foods are added to provide the total MGI ($MGI = MGI_a + MGI_b + MGI_c + MGI_d$) [121]. Empirical evidence has tended to support the efficacy of Wolever & Jenkins methods, demonstrating that the differences in the observed glycemic responses to mixed meals can be predicted by the glycemic indices of the component foods provided the meals contain equivalent amounts of CHO, fat and protein [121-125]. Conflicting evidence however has also been reported with the addition of comparable quantities of fat and protein to different types (but the same amount) of CHO containing foods, spanning a range of glycemic responses (in accordance with their published GI) eliciting identical plasma glucose responses, thus attenuating any of the expected differences [118,126-129]. Methodological differences between laboratories however appears to have contributed to the conflicting data reported.

2.6.2: GI methodological debate

(i) Area under the curve (AUC): GI methodology calculates the incremental area under the glycemic-response curve (iAUC), ignoring any area below fasting levels [87]. Debate exists regarding the validity of disregarding this portion of the circulating blood glucose pool, as all glucose molecules are identical, exert the same metabolic effects and circulate similarly in the blood [130]. Total area under the glucose response curve (tAUC) rather than iAUC has been advocated by some as a more valid measure of glucose availability [113,131]. Using tAUC compared to iAUC greatly diminishes differences in the GI between foods [130]. An alternative view however is that since glucose homeostasis is regulated around ~5 mmol and only the acute ingestion of food results in excursions

above this, eliciting a glucoregulatory response, it is only the incremental area that is of any real significance [113]. Furthermore, since tAUC is an absolute value, while iAUC is relative, the use of the latter enables greater standardization across individuals when comparing the glycemic effects of different foods [121]. Another matter for consideration when calculating iAUC is whether it is valid to exclude negative areas below baseline as the GI protocol advocates. LGI foods have often been observed to result in negative values [131]. Given the acute ingestion of foods can result in blood glucose concentrations both above and below baseline, why then, would the net iAUC (including both positive and negative areas) not be a more accurate depiction of the glycemic effect of a given food?

(ii) Standard duration: Determination of the GI is typically performed over a standard duration of 120 minutes in healthy individuals and 180 minutes for diabetic patients [131]. Disposal of exogenous CHO during the postprandial period and restoration of euglycemia following the acute ingestion of food often takes in excess of this, especially in individuals with diabetes mellitus. This is particularly true following the consumption of slowly digested foodstuff and in diabetes, which does not significantly affect the rate of digestion and absorption but does prolong the postprandial elevation in blood glucose due to an impaired ability to dispose of an exogenous CHO load [131-134]. Restoration of serum insulin concentrations to basal levels can require even longer: 180 minutes in healthy individuals and in excess of 360 minutes in diabetic patients following 50 grams of glucose [132-134]. Some authors have subsequently favored the use of longer observation periods in order to more fully characterize the glycemic and insulinemic effects of different foods [130,131]. The ability of the GI as a marker of CHO quality using

iAUC is based upon distinctions in the early postprandial glycemic response (2-3 hours). For example HGI foods elicit the majority of their glycemic effect early as they result in higher peak but short lived rises in blood glucose concentrations, while LGI foods elicit a relatively smaller rise but sustained increments above baseline due to their slower rate of digestion and absorption. Calculating iAUC over a longer period than 120 minutes in healthy individuals diminishes these earlier postprandial glycemic distinctions [87]. Given the available CHO load provided in GI determination between foods is identical, if iAUC is calculated until all the exogenous CHO is disposed of and basal blood glucose concentrations are restored, almost identical iAUC would be attained. This would appear to negate one of the primary purposes behind the GI concept, which was to relate differences in the rate of digestion and absorption between different foods to their glycemic effects, which are most distinct in the early postprandial period (~ 120 minutes).

(iii) Blood sampling procedure: The GI methodology measures the glycemic responses in capillary blood because of the relative ease and non-invasive nature of this method of blood sampling [87]. While not necessarily controversial this has led to difficulties in comparing glucose data between studies as many have employed venous blood sampling from an antecubital vein [125,127,129,135,136] as opposed to capillary samples from the finger [110,121,123,125]. Venous blood typically yields lower blood glucose concentrations compared to capillary samples, specifically when calculating iAUC, due to capillary blood being arterialized and the possibility of glucose being removed by the skin and skeletal muscle in the forearm [122,131,137]. The use of venous rather than capillary blood has also been shown to greatly diminish differences in the GI between foods [138]. While it could be contended that venous blood represents a more valid depiction of the

overall physiological effect of a given food, such methodological differences from standard GI protocol appears to have confounded some attempts to evaluate the GI's utility between laboratories [110,126,127].

(iv) Individual versus mean responses: A significant portion of debate regarding the GI's clinical utility can also be attributed to differences in analytical techniques employed between laboratories. Jenkins and Wolever at the University of Toronto, calculate the mean GI of test meals as well as the mean plasma glucose response for all individuals, pooling the data and essentially obtaining one data point for the patient population [87,139]. Using this method of data interpretation they were able to demonstrate a strong correlation between predicted and observed GI responses and also in the previous studies by Nutall et al and Bantle et al whose conclusions originally questioned the GI's predictive ability to mixed meals [127,128]. Coulston and coworkers however, contend that such methods are erroneous, preferring to use individual and not mean data points in order to account for individual variability when drawing their conclusions [118,121,140].

(v) Amount of CHO: Since the GI is calculated using only 50 grams of a carbohydrate containing test food and a reference, comparing approximately equal quantities of CHO, it serves as a measure of CHO quality but not quantity. Given that CHO loads in a typical diet usually far exceed 50 grams the GI has been criticized for inadequately characterizing the glycemic response to portion sizes exceeding this amount or whole diets [130]. Since the glycemic effect of a diet is a product of both the CHO quality and quantity, the glycemic load (GL) was introduced in an attempt to more accurately quantify the overall glycemic response [130,141,142]. The higher the GL, the

greater the expected postprandial elevation in blood glucose and insulinogenic demand [111].

$$GL = \frac{\text{GI* grams of available CHO}}{100}$$

While the GL appears to have greater applicability to whole diets than the GI in predicting the glycemic and insulinemic effects, it has been criticized as the GL increases linearly with CHO load, while the amount of CHO consumed has been shown to exhibit a non-linear relationship with glycemic response [143].

2.7: Acute physiological responses to the GI and GL

2.7.1: Metabolic and hormonal effects of the GI: The majority of initial studies investigating the acute metabolic response to consuming foods of differing GI only determined the glycemic responses to single foods [87,110,112,120] and mixed meals [121,122]. The efficacy of prescribing diets using the GI as a therapeutic tool in the prevention and management of chronic diseases however, is contingent not on the glycemic response but changes in underlying metabolic risk factors during the postprandial period, primarily circulating levels of insulin. Furthermore, the health benefits of a GI based diet are contingent upon long-term physiological response (the manifestation of disease), being the chronic sum of acute postprandial perturbations.

Relatively few studies have characterized the postprandial insulinemic response to single GI foods as it was largely assumed that the glucose and insulin responses are directly proportionately to one another, and as such the former could accurately predict the latter. Similar postprandial glycemia however can occur but with markedly different

insulin efficiency and counter-regulatory hormonal responses. Increasingly studies investigating the acute metabolic effects of the to foods of different GI have also measured the insulinemic response, leading to the conception of the insulinemic index (II) a tool for ranking the postprandial insulin responses to foods in the same manner as the GI [125].

$$\mathbf{II} = \left(\frac{\textbf{Incremental plasma insulin area of test food (2 hrs)}}{\textbf{Incremental plasma insulin area of reference food (2 hrs)}} \right) \textbf{x } 100$$

As the GI of a food relates to the rate with which it is digested and absorbed the following discussion will focus on different methods that have been employed to slow the rate of digestion and absorption of CHO foods and their effects on the postprandial gluco-regulatory response along with empirical evidence directly using the GI.

The additions of guar and pectin, both viscous dietary fibres to orally administered CHO solutions and solid feedings, significantly attenuates the postprandial glycemic and insulinemic responses in both healthy and diabetic individuals [144-148]. The effects of viscous fiber on gastric inhibitory polypeptide (GIP) (potentiates insulin secretion) and enteroglucagon (delays gastric emptying, glycogenolytic effects) responses remain less clear [144-148]. Trinick et al fed six healthy subjects two drinks both containing 50 grams of glucose in 700 ml of water, but with 22.3 grams of guar added to one solution. The guar solution resulted in significantly lower postprandial glycemic and insulinemic responses [148]. No differences were observed in GIP, glucagon or cortisol concentrations between the two trials, however. Rate of appearance of exogenous CHO across the gut was also significantly lower following the guar solution, although no differences in endogenous glucose production (EGP) were observed [148].

Increased meal frequency has also been used as a model for mimicking the slow digestion of LGI foods. Continuous sipping (over 240 minutes, thereby increasing meal frequency) compared to single bolus consumption (within 5 minutes) of a 50g glucose solution has been shown to dramatically attenuate the postprandial glycemic and insulinemic responses [149]. Consistent results have also been reported elsewhere using high frequency-low volume nibbling versus a low frequency-high volume gorging protocols [149-152]. Multiple sips resulted in significantly flatter (lower but more sustained) FFA, C-peptide, GIP, glucagon, and enteroglucagon responses over the 240-minute observation period [149]. During the first 2-hours, glucose, insulin, C-peptide (30 and 60 minutes), GIP and enteroglucagon (30, 60 & 120 minutes) were all greater, while branch chained amino-acids (BCAA) were lower (60 minutes) following the single bolus feeding. For the latter 2-hours the trend was reversed as glucose, insulin (180 minutes), C-peptide, GIP (240 minutes) were all higher, while FFA, BCAA, growth hormone, glucagon (180 and 240 minutes) and enteroglucagon (240 minutes) were all lower during the continuous sipping [149]. The single bolus feeding also resulted in greater total urinary catecholamine excretion, which in addition to the heightened glucagon, enteroglucagon and GH concentrations observed during the late postprandial period (2-4 hours) suggest a greater counter-regulatory response compared to the continuous sipping protocol, likely due to the greater perturbations in glycemia observed [149]. Slowing the rate of absorption by continuous sipping resulted in a lower but prolonged insulinogenic effect (greater insulin economy and sensitivity), mirroring the pattern of exogenous CHO appearance (supported by the greater concentrations of C-peptide and GIP at 240 minutes). The sustained suppression of FFA release and lower levels of counter-regulatory hormones

during the late postprandial period compared to the single bolus group support this theory [149]. However, while the findings of Jenkins & coworkers offers support for the metabolic benefits of slowing the rate of digestion and absorption using the food frequency model, it remains to be established as whether the metabolic effects of consuming multiple small volume meals is akin to consuming a single bolus of LGI foodstuff.

A plethora of studies have also investigated the effects of CHO type on postprandial metabolic responses. In healthy individuals, Behall et al also demonstrated a significantly lower glycemic and insulinemic responses following the consumption of starch crackers consisting of 70% amylose (straight chained); 30% amylopectin (branch chained) compared to 30% amylose; 70% amylopectin [80]. The authors suggested that the observed differences were due to a higher rate of digestion and absorption following consumption of starch crackers high in branch-chained amylopectin due to a greater surface area for amylase attack compared to the crackers high in straight-chained amylose [80]. Other investigations have compared the postprandial effects of simple and complex carbohydrates and between different simple sugars. Swan et al observed the postprandial responses to 100 grams of starch, sucrose or glucose. The blood glucose and serum insulin responses were greatest following glucose ingestion compared to both sucrose and starch and following sucrose ingestion when compared to starch only [114]. Lee & Wolever investigated the effects of consuming 25, 50 and 100 grams of simple sugars, glucose (GI = 100), sucrose (GI = 68) and fructose (GI = 19) in a 100 ml solution [111,143]. The magnitude of the glycemic and insulinemic responses was greatest after glucose ingestion followed by sucrose and fructose. With increased CHO intake however, the

insulinemic response increased to a greater extent (linear dose response relationship) than the glycemic response (flattened dose-response relationship above 50g of CHO) [143].

Most studies investigating the acute metabolic effects of the GI have characterized the gluco-regulatory responses to mixed meals. The predictive capabilities of the GI in this context appear greatest in healthy individuals with HGI compared to LGI mixed meals consistently demonstrating greater glycemic and insulinemic postprandial responses [123,135,136,153-158] while less consistently lower postprandial FFA and glycerol concentrations [155,156,157]. Chew et al examined the validity of GI in predicting the glycemic and insulinemic responses to isocaloric ethnic meals of mixed but matched macronutrient content in healthy individuals. Significant correlations were reported between the observed and calculated GI's ($r = 0.88$) along with the observed GI's and II's of the test meals ($r = 0.83$) supporting the GI's utility in this population [123]. The ability of the GI to predict the glycemic and insulinemic (most consistently) responses in diabetic patients appears less clear [125,129,141].

Fewer studies have examined the counter-regulatory hormonal responses to foods of differing GI. Ludwig et al observed the postprandial gluco-regulatory responses in obese healthy boys fed mixed LGI, MGI or HGI meals for breakfast over 300 minutes [135]. Areas under the glycemic and insulinemic response curves were significantly higher following HGI compared to LGI. HGI resulted in greater counter-regulatory responses in the late postprandial period with higher concentrations of epinephrine and growth hormone observed at 270 and 300 minutes. The LGI meal elicited a greater area under the glucagon response curve, and higher FFA concentrations after 150 to 270 minutes compared to HGI [135]. Galgani et al also examined gluco-regulatory hormonal responses

over 300 minutes but in obese healthy females fed small and large GI meals. Areas under the glycemic and insulinemic responses were significantly greater following HGI than LGI with the large meals only [136]. Serum FFA was higher at 240 and 300 minutes following the HGI compared to LGI (large meals only). Contrary to the observations of Ludwig and coworkers however, no differences were reported in plasma glucagon responses based on GI [136]. Differences between the two studies that may account for the inconsistency in glucagon responses reported include; (i) the greater amount of energy provided in the large test meals by Galgani et al compared to Ludwig (3200 kJ; 3.92-4.18 kJ/g versus 1650 kJ; 2.46-2.52 kJ/g) and (ii) the identical macronutrient composition in the HGI and LGI test meals used by Galgani et al, compared to differing macronutrient proportions in Ludwig and Coworkers test meals (64, 20 and 16% in the HGI versus 40, 30 and 30% in the LGI of CHO, fat and protein respectively) [135,136]. To date, fewer studies have observed the counter-regulatory responses to mixed meals of differing GI in healthy individuals. Wee et al fed healthy runners isocaloric LGI and HGI breakfasts (3430 kJ) of identical macronutrient composition (87.5%, 2% and 10.5% of CHO, fat and protein respectively), and observed the proceeding postprandial responses at rest for 180 minutes [157]. The HGI meal resulted in greater glycemic and insulinemic responses but lower FFA concentrations compared to LGI. Glycerol also tended to be lower following HGI than in LGI (non-significant). Consistent with Ludwig et al the LGI test meal also resulted in greater postprandial glucagon responses [157]. Such observations corroborate previous observations from the same laboratory, regarding the effects of the GI on glycemic and insulinemic responses along with circulating concentrations of FFA and

glycerol (significant). Serum cortisol was also determined in this particular study although no differences were observed based on GI [156].

Ludwig et al recently provided a conceptual model to distinguish between the acute postprandial physiological effects of HGI and LGI foods [159]. During the first two hours following the consumption of rapidly digested HGI meals blood glucose is elevated (~30 minutes in healthy individuals) by up to twice the magnitude of the rise observed following LGI foodstuffs [135,136,153,156,157] (Figure 2.3) This transient hyperglycemia occurs in the presence of greatly augmented concentrations of gut incretins (GIP) compared to LGI foods (due to HGI foods higher rate of digestion), which potentiate insulin secretion from beta cells and inhibit glucagon release from pancreatic alpha cells [149,150]. The resultant higher insulin/ glucagon ratio following HGI meals exaggerates the anabolic responses in insulin-responsive tissues including nutrient (glucose) uptake, glycogenesis, lipogenesis and proteogenesis whilst suppressing the catabolic actions of glycogenolysis, gluconeogenesis and lipolysis [150]. Due to the lower hyperglycemic and insulinogenic effect of more slowly digested LGI feedings during this early postprandial period (lower GIP), the anabolic effects of insulin are somewhat attenuated, allowing continued secretion of glucagon (greater concentration than in HGI foods) from pancreatic alpha cells. Glycogenolytic and gluconeogenic HGP along with lipolysis remain closer to basal levels compared to HGI foods evidenced by a lower insulin/ glucagon ratio and higher concentrations of circulating FFA and glycerol during this early postprandial period [135,149,156,157]. Two to four hours following the consumption of HGI meals absorption of exogenous foodstuff across the gut diminishes, but the physiological effects of a high insulin/ glucagon ratio persist (increased cellular glucose

uptake and suppression of EGP) resulting in blood glucose and FFA concentrations falling below basal levels [159] (Figure 2.3). Following LGI meals, continued absorption of nutrients across the gut during the mid-postprandial period results in better maintenance of blood glucose (sustained euglycemia) which in the presence of continued elevations in gut incretins (GIP) continues to maintain serum insulin above basal levels. The lower insulinogenic effect of LGI foods continues to maintain a lower insulin/ glucagon ratio resulting in higher levels of HGP, lipolysis and concentrations of circulating FFA and glycerol during this mid-postprandial period compared to HGI meals [149,150,156,157]. Four to six hours after a HGI meal, low concentrations of blood glucose (hypoglycemia) and FFA trigger a counter-regulatory hormonal response resulting in increased concentrations of glucagon, epinephrine, and growth hormone which stimulate the augmentation of HGP and lipolysis to restore euglycemia and increase circulating levels of FFA [135,149] (Figure 2.3). Following a LGI meal as gut absorption of nutrients declines and serum insulin returns to basal levels, concentrations of counter-regulatory hormones increase slightly. The lower insulinogenic effects of LGI foods and any residual effects on insulin-sensitive tissue, enable a smoother transition from the late postprandial to the early post-absorptive period. HGP gradually increases to maintain euglycemia, while increased levels of lipolysis gradual elevate serum FFA above basal levels [135,159].

2.7.2: Metabolic and hormonal effects of the GL: Less evidence exists characterizing the gluco-regulatory responses to acute feedings using the GL, which is considered a better indicator of the insulinogenic demand of a meal, given that it accounts for both CHO quality (GI) and quantity (available CHO in grams). Brand-Miller et al

tested 10 foods in healthy volunteers (n = 30) and found that identical GL's elicited similar glycemic responses in 9 of the 10 foods [160]. A linear relationship was observed between GL and $iAUC_{glucose}$ which was particularly strong at lower but less so at higher GL's. GL also exhibited a directly proportional relationship to $iAUC_{insulin}$, indicating the GL is an excellent predictor of insulinogenic demand. While some studies comparing the validity of the GI and GL in predicting the glycemic response to test foods have questioned both distinctions validity, their findings have been questioned based on methodologically criticisms, namely; (i) failure to adhere to standard GI protocol; (ii) inaccurate analytical methods used; and (iii) inappropriate selection of GI values [161-163]. Subsequent studies comparing the validity of the GI and GL have offered further support. Galgani et al fed test meals of different GI and GL to obese healthy females and found that while both were good predictors of the glycemic and insulinemic responses, the GL better corresponded to $iAUC_{glucose}$ and $iAUC_{insulin}$ than GI [136]. Neither GI or GL resulted in differences in the plasma glucagon response. The findings of Wolever et al further substantiate this data, observing that the GL explained 90% of the variation in glycemic responses between 14 test meals, compared to 36% with the GI. The GL also accounted for a significant portion of differences in insulinogenic demand between test meals, whereas the GI did not [158].

2.8: Sports nutrition application of the GI

Sports nutrition guidelines for prolonged moderate intensity exercise where the depletion of endogenous CHO is a limiting factor in exercise performance have previously only recommended the optimal amount and timing of CHO consumption with

the aim of maintaining CHO availability to the muscle and central nervous system (CNS) [164,165]. More recently the type of CHO an athlete should eat has been studied, with the GI, as a marker of CHO quality relating to the rate of digestion and absorption suggested as a useful tool in order to manipulate CHO consumption before, during and after exercise to optimize CHO availability [78,164,165]. LGI foods, with their slower rates of digestion are advocated in order to promote sustained CHO availability, whereas HGI foods, due their faster rate of absorption are suggested when a rapid supply of CHO energy is required [78,164]. The GI of a number of common commercial sports nutrition products (sports drinks, energy bars, meal replacement drinks) have been determined, although further documentation of the GI of many others have yet to be ascertained or released into the public domain [111,166,167]. The validity of GI values for such products appears greater than for mixed meals consisting of single foods as criticisms' pertaining to factors that may alter the rate of digestion and absorption is negated given their availability in a readily consumable form.

2.9: Pre-exercise CHO meal (3-4 hours before)

A high carbohydrate meal providing approximately 200-300g of CHO 3-4 hours pre-exercise is considered essential for endurance performance based on observations that an overnight fast dramatically depletes liver glycogen stores [56,165,168,169]. Feeding during this period allows adequate time for the restoration of basal fasting blood glucose and insulin concentrations prior to the commencement of exercise [56,170]. Empirical evidence investigating the efficacy of this nutritional strategy has demonstrated CHO rich meals to augment total CHO metabolism, suppress fat oxidation during exercise whilst improving

work output, endurance running capacity and delaying the onset of fatigue [170-172]. The efficacy of such feedings on the metabolic response to exercise however appears to be contingent on the CHO load consumed. Sherman et al investigated the effects of CHO feedings of 45g, 156g and 312g (0.6g, 2.0g and 4.5g of carbohydrate/ kg of body mass respectively) 4 hours prior to endurance exercise [172]. Findings demonstrated a proportional relationship between the amount of CHO consumed prior to exercise and total CHO oxidation during exercise along with an inverse association between CHO load and the blood glucose, plasma FFA and glycerol response during the first 40 minutes of exercise. Maintenance of euglycemia and improvements in endurance performance however were only observed following the consumption of the 312g of CHO [172].

Mechanistically the augmentation of CHO oxidation and delayed onset of fatigue following a pre-exercise meal is thought to be a consequence of increased muscle and liver glycogen stores along with increased blood glucose availability [170]. Coyle et al observed a 42% increase in muscle glycogen 4 hours following the consumption of 140 g of CHO (2.0g of CHO/ kg of body mass) demonstrating that a large amount of a high CHO meal is disposed of as muscle glycogen [170]. Findings also revealed a significant increase in CHO oxidation during exercise following pre-exercise feedings, which was largely attributable to an increased reliance on muscle glycogen [173,174]. Wright et al however suggests that an increased dependence on muscle glycogen cannot be the sole explanation for the magnitude of enhanced CHO oxidation often observed, based on approximations of the maximal amount of glycogen synthesized 3 hours postprandially [171]. While some liver glycogen may be synthesized during this rest period, estimates of gastric emptying also suggest that the process of digestion and absorption is still in

progress 3-4 hours later, upon exercise commencement. Consequently the absorption of exogenous carbohydrate is thought to provide a key ancillary source facilitating the maintenance of euglycemia and compensating for the augmentation of peripheral glucose extraction from the circulating blood pool [171,172,175].

Despite normalization of blood glucose and insulin prior to exercise however carbohydrate feeding 3-4 hours prior to endurance activity has been shown to result in transient hypoglycemia along with a reduction in plasma FFA and glycerol concentrations most potently during the first hour of exercise [171,172]. Coyle et al outlines that such observations indicate that the effect of postprandial hyperinsulinemia on insulin sensitive tissues persists long after insulin concentrations return to basal levels [170]. Montain et al demonstrated that it takes as long as 6 hours for the normalization of CHO oxidation, indices of lipolysis and plasma glucose homoestasis during exercise following a CHO meal [176]. The persistence of insulin's actions are thought to mediate the reduction in circulating blood FFA and glycerol concentrations observed during the first hour of exercise via its antilipolytic effects on adipose tissue. The transient hypoglycemia observed during the first 20 minutes of exercise also appears consistent with the additive effects of insulin and contraction mediated pathways enhancing peripheral glucose disposal [170]. Insulin is known to suppress HGP so a failure to maintain euglycemia appears to be the result of an imbalance between enhanced peripheral glucose uptake and attenuated splanchnic output [38,170].

Few studies have compared the effects of the GI of pre exercise meals on the metabolic and hormonal response during endurance performance. Wee et al compared the effects of consuming isocaloric, CHO matched high glycemic index (HGI) and low

glycemic index (LGI) meals (solid) 3 hours prior to prolonged exercise [156]. Findings demonstrated that compared with the HGI meal, ingestion of the LGI meal resulted in a reduction in CHO oxidation and an augmentation of fat oxidation during exercise. The differential metabolic effects between the HGI and LGI meals on substrate usage during endurance exercise, was considered a consequence of the postprandial insulinemic response [156]. Reduced hyperinsulinemia following the LGI meal attenuates the persistent effects of pre-exercise insulin mediating the transient hypoglycemia, augmentation of CHO metabolism and the suppression of fat oxidation observed during exercise in the HGI trial [156]. Thorne et al postulates that LGI meals may contribute to better maintenance of euglycemia during endurance exercise based on assumptions that a greater amount of the meal would remain in the gut at the onset of exercise and result in the more gradual absorption of CHO across the intestine compared to HGI meals [81]. Despite differences in substrate usage however during exercise, the contention that such metabolic effects may benefit endurance performance were not supported by the findings from this or later studies [156,177]. Consequently the choice of CHO with regard to GI when planning the pre-exercise meal 3-4 hours prior to exercise appears to be of neglible importance [156].

2.10: Pre-exercise CHO feedings (0-60 minutes before)

CHO intake during the hour prior to prolonged exercise remains a topic of controversy based on the conflicting observations of studies investigating the potential metabolic and performance benefits of pre-exercise feeding during this period. The majority of studies have involved the ingestion of single monosaccharide solutions,

primarily glucose and fructose 60, 45, 30 or in the 15 minutes prior to exercise and not so-called 'real foods', meals or meal replacements [177-181]. A large portion of empirical evidence evaluating the efficacy of consuming glucose solutions (HGI) in this window prior to prolonged high intensity endurance exercise suggests adverse physiological affects. Table 2.4 summarizes the evidence to date regarding the consumption of HGI solutions during this pre-event nutritional window. Studies have consistently shown HGI glucose solutions ingested during the hour prior to exercise, regardless of the precise time (60, 45, 30 or 15 minutes prior), or amount (1g/kg, 50g, 75g) to elicit hyperglycemia result in hyperinsulinemia at the commencement of exercise [177-181]. The consequence of exercising whilst hyperinsulinemic results in the synergistic interaction between insulin and contraction induced pathways drastically augmenting peripheral glucose uptake and resulting in transient hypoglycemia for the initial portion of exercise, although this appears to be corrected during the later stages of prolonged activity [177-181]. The antilipolytic actions of insulin following glucose ingestion have also been shown to suppress adipose tissue lipolysis as evidenced by consistent and significant reductions in circulating FFA and glycerol concentrations. Consequently CHO oxidation has been shown to increase. Some authors postulate that this shift in substrate use during exercise would favor greater rates of muscle and liver glycogen degradation (specifically during the first portion of exercise if hypoglycemic), which may detrimentally affect endurance performance [178,179,182,183]. While Levine et al did observe an increase in muscle glycogen degradation compared to control conditions following the consumption of a glucose solution 45 minutes prior to exercise other investigators have failed to confirm such observations [51,183-185]. Evidence as to whether such conditions may precipitate fatigue

during prolonged endurance exercise also remains equivocal. Foster and colleagues demonstrated a significant reduction in cycling time to exhaustion (19%) when subjects ingested a glucose solution 30 minutes prior to exercise compared to control values [178]. Similar observations were reported by Sherman et al [186]. Other studies however while reporting undesirable physiological perturbations have failed to substantiate such an effect [180,187].

Such findings have led to the contention that ingestion of LGI (fructose) solutions during this window prior to endurance exercise might provide preferentially tighter glycemic control, whilst still providing an ancillary source of glucose in order to maintain endurance performance. Table 2.4 summarizes the physiological affects of consuming such solutions in the hour prior to exercise (during exercise only). Evidence comparing the ergogenic benefits of HGI (glucose) and LGI (fructose) sugar solutions suggest that the ingestion of fructose in the 60 minutes prior to exercise results in smaller postprandial perturbations in the glycemic and insulinemic, negating the transient hypoglycemic observed following the ingestion of HGI solutions upon exercise commencement [182,187]. Data regarding whether such smaller metabolic perturbations result in a sparing of endogenous glycogen stores and improvements in endurance performance remains equivocal, although the majority of studies appear to suggest this is not the case. While Levine et al and Hargreaves et al reported reductions in muscle glycogen depletion and usage following fructose ingestion when compared to glucose [186,185], a larger weight of empirical evidence fails to support such observations [51,184,185]. These authors postulate that the inability of fructose to alter glycogen metabolism is most likely due to its slow gastrointestinal absorption and conversion into glucose by the liver along with its

extensive use in hepatic metabolism [188-190]. Differences in the exercise protocols employed, the training status of subjects, along with prior dietary controls between such studies however, makes it inherently problematic to draw any coherent conclusion regarding this area of debate.

Empirical evidence regarding the efficacy of HGI (potato) and LGI meals (lentils) on prolonged endurance performance offers further evidence for the efficaciousness of LGI foods in limiting glycemic and insulinemic perturbations (refer to Table 2.5). Thomas et al observed that the ingestion of an LGI meal (lentils) 60 minutes prior to exercise significantly attenuated the postprandial glucose and insulin response whilst also prolonging exercise time to exhaustion at 65-70% $VO_{2\ max}$ compared with the ingestion of potato, a HGI food [191]. Similar observations were also reported by Kirwan et al following the ingestion of a MGI (moderate glycemic index), high fiber meal when compared to other pre-exercise feeding conditions [192]. The authors postulated the beneficial effects of such foods may be due to the better maintenance of blood glucose and FFA concentrations during exercise, enabling greater fat utilization and the conservation of higher endogenous CHO stores [191,192]. Such an explanation however appears to contradict with the assumption that a high rate of CHO oxidation is obligatory for sustained endurance performance. While smaller metabolic perturbations have also been consistently demonstrated elsewhere following the consumption of LGI compared to HGI foods further investigations have failed to substantiate the previous conclusions of Thomas et al and Kirwan et al of a glycogen sparing and performance enhancing effect [191,192]. Table 2.5 summarizes the findings of studies regarding this issue. Febbraio et al compared the glycemic and insulinemic response along with endurance performance

following the ingestion of a HGI meal (mashed potatoes), LGI meal (lentils) or a placebo meal [193]. Findings confirmed the benefits of LGI nutrition compared to HGI foods in minimizing fluctuations in the postprandial glycemic and insulinemic response. Pre-exercise CHO ingestion however irrespective of the GI had no effect on the rate of muscle glycogenolysis or endurance performance [193]. Later observations by the same laboratory however demonstrated potential benefits of LGI nutrition in attenuating muscle glycogen use during 120 minutes of cycling at 70% of $VO_{2\,max}$ relative to the HGI and control conditions, which appears to contradict their initial observations [194].

2.11: Summary

The glycemic index (GI) is a standardized system of classification for CHO foods based on their postprandial blood glucose response. GI = incremental area under glycemic response curve of 50 grams test food/ incremental area under glycemic response curve of 50 grams reference food*100 [110]. The GI is considered a measure of CHO quality and relates to the rate at which a food is digested and absorbed [87,111]. Given that CHO loads in a diet typically exceed 50 grams the GI has been criticized for inadequately characterizing the glycemic response to portion sizes exceeding this amount or whole diets [131]. Since the glycemic effect of a meal or diet is a product of both the CHO quality and quantity, the glycemic load (GL) was introduced in an attempt to more accurately quantify the overall glycemic response [141,142]. GL = (GI*Grams of available CHO)/ 100.

Studies that have observed the acute resting metabolic events to consuming foods of differing GI, have consistently demonstrated greater glycemic and insulinemic responses and lower free fatty acid (FFA) concentrations following high glycemic index

(HGI) compared to low glycemic index (LGI) meals in healthy [123,153,155,156] but not always diabetic individuals [125,140]. Few studies have determined the acute counter-regulatory hormonal responses based on the GI or GL, which is necessary to fully characterize the postprandial gluco-regulatory stress to these indices. Observations from obese and healthy patients suggest that HGI meals elicit augmented glycemic and insulinemic responses but suppress glucagonemic, FFA and glycerol levels compared to LGI meals during the early postprandial period [135,157]. Evidence also suggests that the GL may be a better predictor of the insulinemic effect of a meal in healthy individuals [136,150]. Few, if any studies have investigated the effects of both the GI and GL on metabolic and gluco-regulatory hormonal responses to meals in healthy individuals.

During prolonged moderate intensity endurance activity there is an augmentation of blood glucose uptake as muscle glycogen is degraded [7,11]. HGP tries to offset this by increasing glucose production initially via glycogenolysis followed by gluconeogenic production of glucose from glucogenic precursors in order to maintain euglycemia [24,25]. The rate of gluconeogenic glucose production however fails to keep pace with peripheral glucose extraction and often results in a significant reduction in the blood glucose pool to the point of hypoglycemia [10,11].

A reduction in blood glucose has been shown to augment the counter-regulatory response, primarily orchestrated via the actions of glucagon, epinephrine, norepinephrine, cortisol and growth hormone resulting in a reduction in CHO oxidation and an augmented reliance upon fat metabolism [3,4,24]. A reduction in CHO metabolism however is thought to reduce glycolytic flux rate, resulting in impaired ATP resynthesis and a reduced energetic supply to the working musculature, and consequent reductions in endurance

performance [43-45]. The provision of exogenous sources of CHO prior to and during exercise in order to maximize endogenous stores along with supplying an ancillary source of glucose (via absorption across the gut) to maintain euglycemia is considered paramount to insure optimal endurance performance [38,48].

The glycemic index (GI) as been suggested as a useful tool for athletes in terms of selecting the most appropriate type of carbohydrate to consume prior to, during and after endurance activity [78,111]. Evidence has shown pre exercise meals 3-4 hours prior to competition should be considered an essential component of an endurance athletes' pre-event nutritional strategy in order to maximize endogenous stores of carbohydrate and maintain euglycemia during competition [56,168]. Such benefits have been observed regardless of the GI [156].

Recommendations with regard to pre-exercise CHO feedings the hour prior to competition however remain an area of controversy and considerable debate. CHO feedings the hour prior to exercise appear to have neglible affects on muscle glycogen depletion and endurance performance. While some have reported reductions in muscle glycogen degradation and improved endurance performance as a result of LGI compared to HGI feedings [183], such observations have failed to be replicated in a plethora of further studies [51,184,185]. Nonetheless LGI feedings the hour prior to exercise, have consistently been shown to result in more favorable glycemic control and smaller metabolic perturbations compared to HGI alternatives [182,189]. Recommendations consequently suggest that if athletes choose to eat the hour prior to competition the consumption of LGI and not HGI foods should be advocated [56]. Findings however, remain equivocal and further investigation appears warranted. Furthermore no studies

appear to have sufficiently characterized the underlying counter-regulatory hormonal responses based on both the GI and GL as a result of this nutritional strategy. Such investigation may help to further elaborate upon the potential differential metabolic effects of foods of contrasting GI/ GL the hour prior to prolonged endurance activity.

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Table 2.1: HGI foods (GI > 70). GI values based on white bread as the reference food (111,166,167)

Vegetables, fruits & juices	Cereals	Snack foods, beverages	Sports products
Cranberry Juice (80)	Cheerios (106)	Coca Cola (83)	Gatorade - orange (111)
Orange Juice (74)	Bran Flakes (106)	Fanta (97)	Lucozade – original (136)
Apricots – raw (82)	Shredded Wheat (107)	Corn chips (90)	Isostar (100)
Banana – raw (74)	Cornflakes (116)	Potato chips – plain, salted (77)	Powerbar - chocolate (79)
Sweet corn (78)	**Grains**	Digestives (84)	Ensure - vanilla (75)
Baked potato (85)	White rice – boiled (91)	Crunchy nut cornflakes bar (102)	Cliff bar - cookies & cream (101)
Instant mashed potato (122)	Brown rice (79)	Mars bar (93)	**Whole meals**
Mashed potato (105)	Instant rice (98)	Snickers bar (78)	Spaghetti Bolognase (74)
French Fries (107)	**Breads**	**Sugars**	Sushi (74)
Sweet potato (87)	Bagel - white (103)	Glucose (141)	Chicken & vegetable stir fry (104)
Sultanas (80)	Baguette – white (136)	Sucrose (97)	Macaroni & cheese – kraft (92)

Table 2.2: MGI foods (GI 56-69). GI values based on white bread as the reference food (111,166,167)

Vegetables, fruits & juices	Cereals	Snack foods, beverages	Sports products
Apple Juice (57)	All bran (60)	White chocolate (63)	Ironman PR bar (55)
Carrot Juice (61)	Muesli (69)	Twix bar (63)	Sustagen sport (61)
Grapefruit Juice (69)	Wheat – whole kernels (59)	Chocolate milk (61)	**Pasta**
Pineapple juice (66)	Parboiled rice (68)	Soy milk – full fat (63)	Instant noodles (67)
Grapes – raw (66)	**Legumes**	Ensure bar – chocolate, fudge (61)	Spaghetti, white – boiled (60)
Oranges – raw (60)	Baked beans (69)	**Bakery Products**	Linguine (65)
Peaches – raw (60)	Black-eyed beans (59)	Sponge cake (66)	Macaroni (67)
Strawberries – raw (57)	Lentil soup (63)	Muffin –apple (69)	Fettucine (57)
Carrots – raw (68)	Minestrone soup (56)	**Breads**	**Whole Meals**
Green peas – boiled (68)	**Sugars**	Oat-bran bread (68)	Beef pies (64)
Marmalade – orange (69)	Lactose (66)	Fruit loaf (63)	Chicken nuggets (66)

Table 2.3: LGI foods (GI < 55). GI values based on white bread as the reference food (111,166,167)

Vegetables, fruits & Juices	Cereals	Snack Foods & Beverages	Sports Products
Tomato Juice (54)	Barley (36)	M & M's – peanut (47)	Pure protein bars (49)
Apples (52)	Rye – whole kernels (48)	Nutella – chocolate (47)	Solo GI bars (22-29)
Pears – raw (54)	**Legumes**	Smoothie - rasperry (48)	**Whole meals**
Plums – raw (41)	Kidney beans (39)	Milo (51)	Pizza – 11.4% fat (51)
Apple – dried (41)	Lentils – green (42)	Nutrimeal (37)	Hummus (9)
Apricots – dried (44)	Lentils – red (36)	**Dairy products**	Spaghetti, whole meal – boiled (53)
Cherries – raw (32)	Butter beans (43)	Milk – full fat (38)	Sausages (40)
Grapefruit – raw (36)	Chickpeas (39)	Milk – skimmed (46)	Fish fingers (54)
Tomato soup (54)	Soya beans (25)	Yogurt (51)	**Nuts**
Yam (53)	**Sugars**	Custard (54)	Cashew nuts (31)
	Fructose (27)	Mousse (48)	Peanuts (21)

Table 2.4: Summary of reported changes to blood borne-substrates, hormonal responses, muscle glycogen, substrate oxidation during exercise and endurance performance following the ingestion of CHO solutions of differing glycemic index the hour prior to exercise. All trials are compared with control/ placebo conditions. Arrows denote an increase (↑), decrease (↓) and no change ↔. * indicates the magnitude of the observed change was greater when compared to fructose. CPT = constant power (intensity) test; CDT = constant duration test; CWT = constant work test.

PRE-EXERCISE MEAL TIMING	STUDY	AMOUNT & TYPE OF CHO	EXERCISE PROTOCOL	BLOOD GLUCOSE	FFA	GLYCEROL	LACTATE	INSULIN	GLUCAGON	CORITSOL	EPINEPHRINE	NOREPINEPHRINE	MUSCLE GLYCOGEN	RER	ENDURANCE PERFORMANCE
SOLUTIONS															
FRUCTOSE - LGI (23)															
60 min Pre	**Guezennec et al 1989** Compared to glucose only	**1g/ kg**	**Bike CPT 120 min 60% $Vo_{2\,max}$**	↔	↑ ns	↑		↓						↔	
	Decombaz et al 1985 Compared to glucose only	**1g /kg**	**Bike (i) CPT 45 min 61 % $Vo_{2\,max}$ + (ii) CDT 15 min Sprint**	↔	↔	↔	↔	↔	↔				↔	↔	↔
45 min Pre	**Hargreaves et al 1987**	**75g**	**Bike CPT 75% $Vo_{2\,max}$ to exhaustion**	↔			↔	↔					↔	↔	↔
	Koivisto et al 1985	**75g**	**Bike CPT 120 min 55% $Vo_{2\,max}$**	↔	↓		↔	↔		↔			↔		
	Levine et al 1983	**75g**	**Bike CPT 30 min 75% $Vo_{2\,max}$**	↔		↓		↔					↑	↑	

PRE-EXERCISE MEAL TIMING	STUDY	AMOUNT & TYPE OF CHO	EXERCISE PROTOCOL	BLOOD GLUCOSE	FFA	GLYCEROL	LACTATE	INSULIN	GLUCAGON	CORITSOL	EPINEPHRINE	NOREPINEPHRINE	MUSCLE GLYCOGEN	RER	ENDURANCE PERFORMANCE
	Koivisto et al 1981	75g	Bike (i) CPT 30 min 75% $Vo_{2\,max}$+ (ii) CPT to exhaustion	↔	↓		↔	↑		↔					↔
	Hargreaves et al 1985	50g	Bike CPT 30 min 75% $Vo_{2\,max}$	↔			↔						↔	↔	
30 min Pre	**Ventura et al 1994**	75g	Treadmill CPT 82% $Vo_{2\,max}$ to exhaustion	↔			↔	↑ ns						↔	↑ ns
	Fielding et al 1987	75g	Treadmill CPT 30 min 70% $Vo_{2\,max}$	↔			↔						↔	↔	
	Calles – Escandon et al 1991	65g	Bike CPT 75% $Vo_{2\,max}$ to exhaustion	↔	↔		↔	↔						↔	↔
GLUCOSE - HGI (100)															
60 min Pre	**Guezennec et al 1989** Compared to fructose only	1g/kg	Bike CPT 120 min 60% $Vo_{2\,max}$	↔	↔	↑		↑						↔	
	Decombaz et al 1985 Compared to fructose only	1g/kg	Bike (i) CPT 45 min 61 % $Vo_{2\,max}$ + (ii) CDT 15 min Sprint	↔	↔	↔	↔	↔					↔	↔	↔
	Thomas et al 1991	1g/kg	Bike CPT 65-70% $Vo_{2\,max}$ exhaustion	↔	↓		↔	↔						↑	↔

PRE-EXERCISE MEAL TIMING	STUDY	AMOUNT & TYPE OF CHO	EXERCISE PROTOCOL	BLOOD GLUCOSE	FFA	GLYCEROL	LACTATE	INSULIN	GLUCAGON	CORITSOL	EPINEPHRINE	NOREPINEPHRINE	MUSCLE GLYCOGEN	RER	ENDURANCE PERFORMANCE
45 min Pre	Hargreaves et al 1987	75g	Bike CPT 75% $Vo_{2\,max}$ to exhaustion	↓ *			↔	↑*					↔	↔	↔
	Gleeson et al 1986	1g /kg	Bike CPT 73% $Vo_{2\,max}$ to exhaustion	↔	↓	↔	↔	↔						↑	↑
	Koivisto et al 1985	75g	Bike CPT 120 min 55% $Vo_{2\,max}$	↔	↓		↔	↑*		↔			↔		
	Levine et al 1983	75g	Bike CPT 30 min 75% $Vo_{2\,max}$	↓ *		↓		↑*					↔ ↓ *	↑	
	Koivisto et al 1981	75g	Bike (i) CPT 30 min 75% $Vo_{2\,max}$ + (ii) CPT to exhaustion	↓ *	↓		↓ ns	↑*		↔					↔
	Hargreaves et al 1985	50g	Bike CPT 30 min 75% $Vo_{2\,max}$	↓ *			↑ *	↑					↓ * ns	↔	
	Ahlborg et al 1977	200g	Bike CPT 240 min 30% $Vo_{2\,max}$	↑	↓	↓	↓	↑	↓					↑	
30 min Pre	Ventura et al 1994	75g	Treadmill CPT 82% $Vo_{2\,max}$ to exhaustion	↓ *			↔	↑*						↔	↑
	Fielding et al 1987	75g	Treadmill CPT 30 min 70% $Vo_{2\,max}$	↓			↔						↔	↑	

PRE-EXERCISE MEAL TIMING	STUDY	AMOUNT & TYPE OF CHO	EXERCISE PROTOCOL	BLOOD GLUCOSE	FFA	GLYCEROL	LACTATE	INSULIN	GLUCAGON	CORITSOL	EPINEPHRINE	NOREPINEPHRINE	MUSCLE GLYCOGEN	RER	ENDURANCE PERFORMANCE
	Foster et al 1987	75g	Bike CPT 70% $Vo_{2\,max}$ exhaustion	↓	↓	↔	↔							↑ ns	↓
	Chryssant -hopoulos et al 1994	75g	Treadmill CPT 70% $Vo_{2\,max}$ to exhaustion	↔			↔							↔	↑ ns
	Marmy-Conus et al 1996	75g	Bike CPT 60 min 71% $Vo_{2\,max}$	↑	↓		↑	↑	↔		↔	↔		↔	
	Goodpaster et al 1996	1g /kg	Bike (i) CPT 90 min 66% $Vo_{2\,max}$ + CDT 30 min Sprint	↔		↓		↔	↔					↓	↑

Table 2.5: Summary of reported changes to blood borne-substrates, hormonal responses, muscle glycogen, substrate oxidation during exercise and endurance performance following the ingestion of solid carbohydrate nutrition of differing GI the hour prior to endurance exercise. All trials are compared with control/ placebo conditions. Arrows denote an increase (↑), decrease (↓) and no change ↔. * indicates the magnitude of the observed change was greater when compared to LGI nutrition. CPT = constant power (intensity) test; CDT = constant duration test; CWT = constant work test.

SOLID FEEDINGS

LGI (≤ 55)

PRE-EXERCISE MEAL TIMING	STUDY	AMOUNT & TYPE OF CHO	EXERCISE PROTOCOL	BLOOD GLUCOSE	FFA	GLYCEROL	LACTATE	INSULIN	GLUCAGON	CORITSOL	EPINEPHRINE	NOREPINEPHRINE	MUSCLE GLYCOGEN	RER	ENDURANCE PERFORMANCE
60 min Pre	**Thomas et al 1991**	1g/ kg Lentils GI = 29	Bike CPT 65-70% $Vo_{2\,max}$ to exhaustion	↑	↓		↔	↑						↑ ns	↑
45 min Pre	**Sparks et al 1998**	1g/ kg Lentils GI = 29	Bike (i) CPT 50 min 70% $Vo_{2\,max}$ + (ii) CDT 15 min	↔	↓		↔	↔						↔	↔
	Febbraio et al 1996	1g/ kg Lentils GI = 29	Bike (i) CPT 120 min 70% $Vo_{2\,max}$ + (ii) CDT 15 min	↔	↓		↔	↔					↔		↔
30 min Pre	**Febbraio et al 2000**	1g/ kg Muesli GI = 52	Bike (i) CPT 120 min 70% $Vo_{2\,max}$ + (ii) CDT 30 min	↔	↔		↔	↔					↔	↔	↔

PRE-EXERCISE MEAL TIMING	STUDY	AMOUNT & TYPE OF CHO	EXERCISE PROTOCOL	BLOOD GLUCOSE	FFA	GLYCEROL	LACTATE	INSULIN	GLUCAGON	CORITSOL	EPINEPHRINE	NOREPINEPHRINE	MUSCLE GLYCOGEN	RER	ENDURANCE PERFORMANCE
MGI (56-69)															
45 min Pre	Kirwan et al 1998	75g Oats GI = 60-70	Bike (i) CPT 60% $Vo_{2\,max}$ to exhaustion	↔	↓	↔	-	↔			↔	↔	↔	↔	↑
HGI (≥ 70)															
60 min Pre	Thomas et al 1991	1g/ kg Baked Potato GI = 98	Bike (i) CPT 65-70% $Vo_{2\,max}$ to exhaustion	↑	↓		↑* ns	↔						↑*	↔
45 min Pre	Sparks et al 1998	1g/ kg Mash Potato GI = 80	Bike (i) CPT 50 min 70% $Vo_{2\,max}$ + (ii) CDT 15 min	↔	↓ *		↔	↑						↑*	↔
	Febbraio et al 1996	1g/ kg Mash Potato GI = 80	Bike (i) CPT 120 min 70% $Vo_{2\,max}$ + (ii) CDT 15 min	↔	↓ *		↔	↔					↔	↑	↔
30 min Pre	Febbraio et al 2000	1g/ kg Mash Potato GI = 80	Bike (i) CPT 120 min @ 70% $Vo_{2\,max}$ + (ii) CDT 30 min	↔	↓ *		↔	↔							↔
UNDISCLOSED GI															
30 min Pre	Devlin et al 1986	43g Candy Bar	Bike (i) CPT 15 min work 70% $Vo_{2\,max}$ 5 min rest (W:R = 3:1) to exhaustion	↑ ns	↔	↑ ns	↔	↑ ns					↔	↔	↔

PRE-EXERCISE MEAL TIMING	STUDY	AMOUNT & TYPE OF CHO	EXERCISE PROTOCOL	BLOOD GLUCOSE	FFA	GLYCEROL	LACTATE	INSULIN	GLUCAGON	CORITSOL	EPINEPHRINE	NOREPINEPHRINE	MUSCLE GLYCOGEN	RER	ENDURANCE PERFORMANCE
	Calles – Escandon et al 1991	43g Candy Bar	Bike (i) CPT 75% $Vo_{2\,max}$ to exhaustion	↕	↕		↕	↕						↕	↕

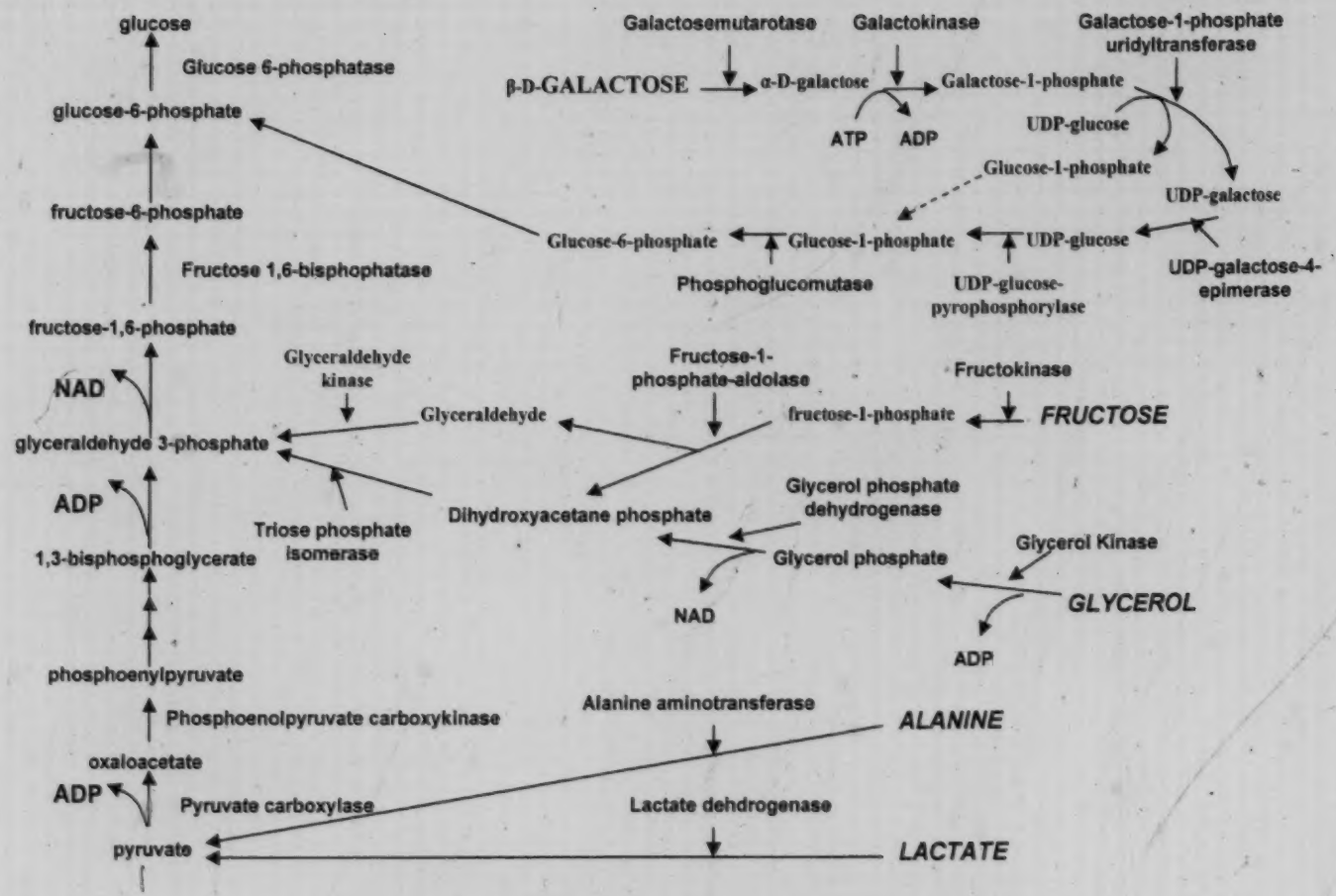
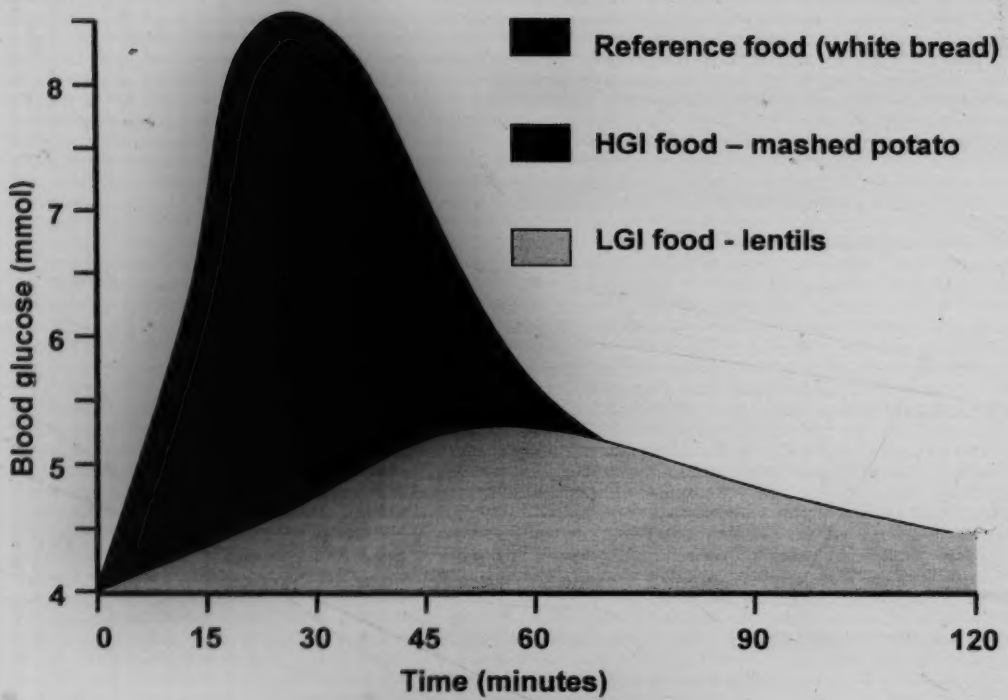


Figure 2.1: Glucogenesis from non-glucose sources



Time (min)	Blood glucose (mmol/l)
0	0
15	2.124
30	0.95
45	-0.16
60	-0.664
90	0.765
120	-0.589

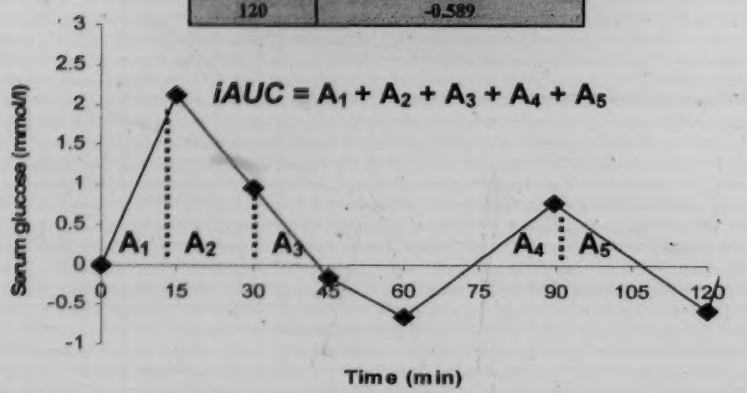


Figure 2.2: Calculating the GI of a test food. iAUC = incremental area under the curve.

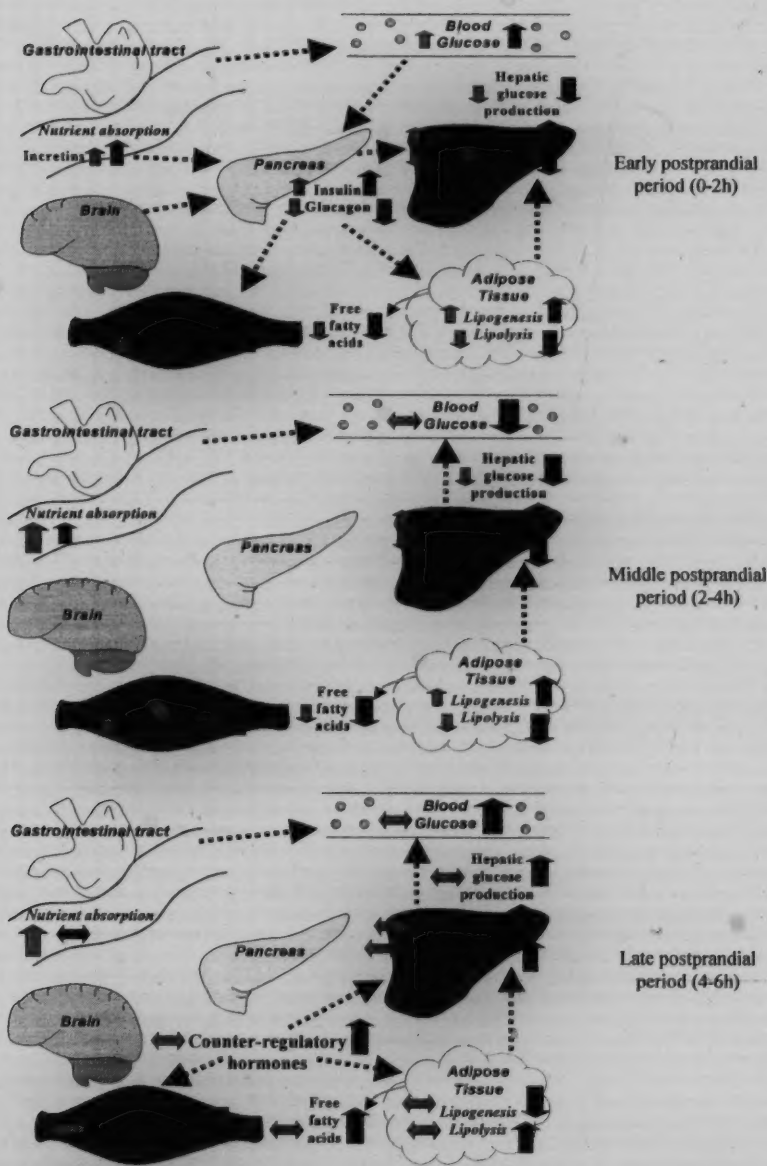


Figure 2.3: Early postprandial: rapid absorption of CHO after HGI ⬆ compared to LGI meal ⬇ results in blood glucose spike and high insulin/ glucagon ratio. Middle postprandial: transient hypoglycemia, continued attenuation of FFA availability following HGI meal. Late postprandial: counter-regulatory hormones restore euglycemia and augment FFA availability. Adapted from Ludwig (2002).

CHAPTER 3 – STUDY ONE

The effect of the glycemic index and load on the glucose and gluco-regulatory hormonal response at rest.

ABSTRACT

The aim of this study was to examine the effects of the GI and GL on the metabolic and gluco-regulatory hormonal responses at rest. Twelve male subjects ingested low glycemic index/ load (LGI/ LGL) and high glycemic index/ moderate glycemic load (HGI/ MGL) nutrition bars providing 1g CHO per kg body mass and a moderate glycemic index/ high glycemic load (MGI/ HGL) nutrition bar in an isocaloric amount to the LGI/ LGL trial while at rest. Insulinemic responses during LGI/ LGL were lower ($P < 0.05$) compared to HGI/ MGL and MGI/ HGL. Serum non-esterified fatty acid (NEFA) concentrations at 120 and 150 minutes during MGI/ HGL were lower ($P < 0.05$) than LGI/ LGL and HGI/ MGL. Total area under the NEFA response curve ($tAUC_{NEFA}$) was greater ($P < 0.05$) in LGI/ LGL and HGI/ MGL compared to MGI/ HGL. Serum glucagon responses during LGI/ LGL were greater ($P < 0.05$) compared to MGI/ HGL. The insulin/ glucagon ratio was lower ($P < 0.05$) at 30 and 45 minutes during LGI/ LGL and HGI/ MGL compared to MGI/ HGL. Consumption of LGI/ LGL foods produce a lower insulin/ glucagon ratio compared to HGL nutrition despite similar glycemic profiles.

Additional information pertaining to study one is in appendices 2, 4, 6, 7 and 10.

INTRODUCTION

The glycemic index (GI) is a standardized system of classification for carbohydrate (CHO) foods based on their postprandial blood glucose response. GI = incremental area under glycemic response curve of 50 grams test food/ incremental area under glycemic response curve of 50 grams reference food*100 [1]. The GI is considered a measure of CHO quality and relates to the rate at which a food is digested and absorbed [2,3]. Given that CHO loads in a diet typically exceed 50 grams the GI has been criticized for inadequately characterizing the glycemic response to portion sizes exceeding this amount or whole diets [4]. Since the glycemic effect of a meal or diet is a product of both the CHO quality and quantity, the glycemic load (GL) was introduced in an attempt to more accurately quantify the overall glycemic response [5,6]. GL = (GI*Grams of available CHO)/ 100. Proponents of the GI and GL advocate their efficacy in the prevention and management of chronic conditions through the prescription of a varied low glycemic diet [7-10]. Debate persists however regarding their clinical utility [2,11-16].

Studies that have observed the acute resting metabolic events to consuming foods of differing GI, have demonstrated greater glycemic and insulinemic responses and lower free fatty acid (FFA) concentrations following high glycemic index (HGI) compared to low glycemic index (LGI) meals in healthy [17-20] but not always diabetic individuals [15,21]. Few studies have determined the acute counter-regulatory hormonal responses based on the GI or GL, which is necessary to fully characterize the postprandial gluco-regulatory stress to these indices. Observations from obese and healthy patients suggest that HGI meals elicit augmented glycemic and insulinemic responses but suppress glucagonemic, FFA and glycerol levels compared to LGI meals during the early

postprandial period [22-24]. Evidence also suggests that the GL may be a better predictor of the insulinemic effect of a meal in healthy individuals [25]. Few, if any studies have investigated the effects of both the GI and GL on metabolic and gluco-regulatory hormonal responses in healthy individuals at rest. Furthermore, all previous studies have used mixed meals to characterize gluco-regulatory hormonal responses based on the GI and GL [17-25]. Controversy exists regarding the GI's efficacy in predicting the glycemic response to mixed meals based on the myriad of factors that affect the magnitude and duration of postprandial glycemia [26-32], including the co-ingestion of fat and protein with CHO [26,28,30,32]. Some studies support the GI's predictive capabilities in this context [11,17,21,32], while others do not [12,13,34-36]. Nutrition bars are a convenient and readily accessible food-source. Given their balanced macronutrient composition and known GI and GL values [3,37,38] they would appear to facilitate more valid investigation into these indices efficacy in predicting postprandial metabolic responses to meals containing significant quantities of CHO, protein and fat. It is also difficult to find studies that have characterized the metabolic and gluco-regulatory hormonal responses to the GI and GL in nutrition bar form at rest.

The purpose of this study was to determine the glucoregulatory hormonal and metabolic responses to nutrition bar feedings of differing GI and GL in healthy individuals at rest, consistent with the GI protocol. The amount of CHO ingested (1g CHO/ kg body mass) was designed to provide a CHO load consistent with an oral glucose tolerance test (OGTT) (50-100 grams). It was hypothesized that, compared with HGI/HGL, ingestion of a LGI/LGL meal of mixed macronutrient composition in a bar form would induce a smaller postprandial glycemic response and insulin/ glucagon ratio.

METHODS

Participants: Twelve healthy males (27.6 ± 3.8 years, 180 ± 10 cm, 83.0 ± 5.5 kg, and body mass index (BMI) = 25.0 ± 2.0 kg/ m^2) volunteered as subjects for this study after being informed of the risks associated with participation and completing a letter of informed consent. The study was approved by the Faculty of Physical Education and Recreation Research Ethics Board at the University of Alberta.

Feeding trials and experimental protocol: Laboratory testing necessitated four separate visits. The study was a within subject design with the trials completed in a randomized order. Prior to commencement of the feeding trials each subject attended the laboratory for an orientation session during which further details of the study were explained, descriptive data was gathered and subjects were familiarized with the testing procedures and equipment. Subjects were asked to record the type and amount of food and beverage consumed in 24 hours during a "typical" day at this time. The 24-hour dietary record was analyzed using a software program (Food Processor II, EHSA, USA) to determine macronutrient content and modified where necessary to establish a percent breakdown of macronutrients of 60% carbohydrate, 15% protein and 25% fat. Subjects were asked to consume identical amounts and types of food specified on their diet record, the day prior to each trial (3116 ± 422.7 kcal which consisted of 60 ± 0.7 % carbohydrate, 15.2 ± 1.3 % protein and 24.2 ± 1.5 % fat).

Subjects reported to the laboratory between 7:00 and 8:00 am on three subsequent occasions, following a 10-hour overnight fast. The 3 trials were separated by at least 5 days, and were conducted in no more than a 4-week period. Subjects were required to abstain from physical activity, alcohol and smoking the day prior to testing. Upon arrival

in the laboratory, subjects body mass was recorded (during the first trial only), an intravenous cathelon was inserted and a fasting blood sample was obtained (0 min). Subjects then consumed 1 of 3 nutrition bar feedings (single blind randomized order) within 10 minutes of their baseline blood sample. The feedings were a low GI (LGI/ LGL 1g CHO/ kg) nutrition bar (GI = 27, GL = 22.35 ± 1.49), a high GI (HGI/ MGL 1g CHO/ kg) nutrition bar (GI = 70; GL = 57.95 ± 3.89), both providing 1-gram of carbohydrate per kilogram of body weight and a moderate GI (MGI/ HGL isocaloric) nutrition bar (GI = 60, GL = 81.27 ± 5.41), calorie matched to LGI/ LGL (1g CHO/ kg). Previous research has demonstrated the effectiveness of 1g/ kg of carbohydrate in significantly altering the blood glucose and hormonal response to different types of carbohydrate foods [39,40]. Both LGI/ LGL (1g CHO/ kg) and MGI/ HGL (isocaloric) provided 827.78 ± 55.05 kcal, while HGI/ MGL (1g CHO/ kg) provided 788.47 ± 52.48 kcal. Subjects were provided with ~ 200 ml of water with the test meals and at 30-minute intervals thereafter. Ten minutes were permitted to consume the test meals, with the actual time taken recorded and subjects asked to replicate this for all proceeding trials. In addition to the fasting blood sample (0 minutes), further samples were obtained 15, 30, 45, 60, 90, 120 and 150 minutes after complete consumption of the test meal. Previous research supports the use of such sample timing in characterizing the postprandial glycemic and hormonal response [2,7]. Figure 3.1 depicts the trial procedure.

Analytic techniques: 5 milliliters (ml) of blood were collected using a 22-gauge cathelon inserted by a registered nurse, and obtained with a syringe. 0.5 ml of sterile saline (0.9% NaCl) was used to keep the cathelon patent during each trial. Prior to obtaining the sample, approximately 1 ml of blood was withdrawn and discarded to

ensure the subsequent sample was not diluted by saline. Whole blood was analyzed immediately for hematocrit in duplicate following centrifugation for 5 minutes in a 50-ul micro centrifuge tube. The remaining blood was allowed to clot (~40 minutes), centrifuged at 1500 xg, with the serum supernatant drawn off and stored at -80° C for later analysis. Glucose was measured using the glucose oxidase method on a spectrophotometer (Sigma-Aldrich USA) while non-esterified fatty acids (NEFA) were determined by a commercially available enzymatic colorimetric technique (Wako Chemicals USA). Insulin, and glucagon concentrations were determined using commercially available radioimmunoassay (RIA) kits (Diagnostic Products Corporation (DPC) USA). All analyses were performed in duplicate. Mean coefficients of variation (CV) for the RIA's were 12.47 and 17.01 % for insulin and glucagon, respectively. In the case of any missing data, for any pre-treatment values, the mean of the other two trials fasting concentration was used, while for any post-treatment (postprandial) data the preceding value was carried forward. The total serum responses were normalized to zero concentration. Incremental serum responses were normalized to fasting concentrations. Total (tAUC) and incremental (iAUC) areas under the curves were calculated geometrically using the trapezium rule, with only the area above the normalized concentrations included in the area calculations [11].

Statistical analyses: Statistica (Statsoft, Oklahamoa) was used for all data analysis. Data were expressed as means ± S.D. A two-way analysis of variance (ANOVA), with repeated measures was used to determine any significant differences between the trials. A Tukey post-hoc test was used to locate differences when the two-way ANOVA revealed a significant interaction. Correlations between protein, fat,

available CHO, GI and GL and mean iAUC for glucose, insulin, glucagon and insulin/ glucagon ratio were also determined. An alpha of $p<0.05$ was considered statistically significant.

RESULTS

No difference was observed in the magnitude of change in hematocrit when comparing the three trials ($P > 0.05$).

A significant main effect of time was observed for the total serum glucose response ($P < 0.05$) (Figure 3.2). The total glucose concentration at 0 minutes was lower than 15, 30 and 90 minutes, while the total glucose concentrations at both 15 and 30 minutes were greater ($P = 0.00$) compared to 45, 60, 90, 120 and 150 minutes. A significant interaction between trial and time was observed with the incremental glucose response ($P < 0.05$). The peak incremental glucose concentration occurred at 15 minutes during all three trials. This value was greater ($P < 0.05$) than concentrations at 45, 60 and 90 minutes in LGI/ LGL trial, 60 and 90 minutes in HGI/ MGL trial, and 45, 60, 90, 120 and 150 minutes in the MGI/ HGL trial. The incremental concentration at 30 minutes was also significantly greater ($P < 0.05$) than 60 minutes in LGI/ LGL and all proceeding time points in MGI/ HGL (Table 3.2).

A significant interaction between trial and time was observed for the serum NEFA responses ($P < 0.05$) (Tables 3.2 and 3.3). Ingestion of the meal during HGI/ MGL resulted in greater ($P < 0.05$) total NEFA concentrations at 120 and 150 minutes and incremental NEFA values for all time points than in MGI/ HGL (Fig 4.3 and 4.4). Incremental NEFA concentrations at 45, 60, 90, 120 and 150 minutes were also greater (P

< 0.05) than in LGI/ LGL, while both serum NEFA concentrations after 90 and 120 minutes were greater ($P < 0.01$) following ingestion of LGI/ LGL compared to MGI/ HGL. During all three trials the peak total NEFA concentration occurred at 0 minutes, which was higher ($P < 0.01$) than all other time points for LGI/ LGL and MGI/ HGL, but excluding 15 minutes for the HGI/ MGL trial. Serum NEFA concentrations at 15 minutes were also greater ($P < 0.01$) than 30, 45, 60, 90 and 120 minutes for LGI/ LGL and MGI/ HGL trials, but excluding 120 and 150 minutes for the HGI/ MGL trial. The incremental NEFA response at 150 minutes during LGI/ LGL was greater ($P \leq 0.01$) than 45 and 60 minutes, while during MGI/ HGL, the incremental response at 30 minutes was higher ($P < 0.05$) than 60, 90 and 120 minutes. The incremental NEFA response at 45 minutes was also significantly lower when compared to both 120 and 150 minutes for HGI/ MGL ($P < 0.05$). A significant main effect of trial was observed in tAUC when comparing the three trials ($P \leq 0.01$), with post hoc analysis revealing a greater ($P < 0.05$) tAUC for both LGI/ LGL and HGI/ MGL compared to MGI/ HGL (Table 3.4).

A significant main effect of trial and time was found for the serum insulin responses ($P < 0.05$). The insulin responses during LGI/ LGL were lower ($P < 0.05$) compared to the HGI/ MGL and MGI/ HGL trials (Figure 3.5). The total insulin concentration at 0 minutes was lower ($P < 0.05$) than values for all other time points, while insulin concentrations after 15 and 45 minutes were greater ($P < 0.01$) compared to 90, 120 and 150 minutes (Figure 3.6). The peak insulin responses occurred at 30 minutes, which were greater ($P < 0.01$) than proceeding total insulin values at 60, 90, 120 and 150 minutes, while insulin responses at 60 minutes were greater ($P < 0.01$) than at 150 minutes. A significant main effect of trial was observed when analyzing areas under the

curve with post hoc analysis revealing both tAUC and iAUC for LGI were lower ($P < 0.05$) compared to HGI/ MGL and MGI/ HGL (Tables 3.4 and 3.5).

A significant main effect of trial was observed for the serum glucagon responses ($P < 0.05$) revealing that the serum glucagon response were greater during the LGI/ LGL trial ($P < 0.05$) compared to MGI/ HGL trial (Figure 3.7). A significant main effect of time was demonstrated ($P < 0.05$) (Figure 3.8). The serum glucagon concentrations were lowest at 0 and 15 minutes, which were both lower ($P < 0.05$) than after 60, 90, 120 and 150 minutes. A significant main effect of trial was observed when analyzing areas under the curve, revealing both tAUC and iAUC for LGI/ LGL were greater ($P < 0.05$) when compared to MGI/ HGL (tables 3.4 and 3.5).

A significant interaction between trial and time was observed with both the total and incremental insulin/ glucagon responses ($P < 0.05$) (Tables 3.2 and 3.3). Ingestion of the meal during MGI/ HGL resulted in greater ($P < 0.05$) ratios at 30 and 45 minutes compared to LGI/ LGL and HGI/ MGL, and at 15 minutes compared to LGI/ LGL only (Figure 3.9). During trial 3, the total and incremental ratio response at 15, 30 and 45 minutes were all significantly greater compared to the proceeding values after 0, 90, 120 and 150 minutes ($P < 0.05$).

Insulinemic iAUC after the test meals was significantly correlated with GI ($r = 0.525$, $p = 0.001$), GL ($r = 0.544$, $p = 0.000$), available CHO ($r = 0.339$, $p = 0.043$), protein ($r = 0.408$, $p = 0.408$) and fat ($r = 0.427$, $p = 0.009$) content (Figure 3.10). Glucagonemic iAUC was significantly correlated with protein content ($r = 0.405$, $p = 0.027$), while insulin/ glucagon ratio iAUC was significantly correlated with both protein ($r = 0.485$, $p = 0.010$) and fat ($r = 0.467$, $p = 0.016$) content (Table 3.6).

DISCUSSION

Few, if any studies have examined the acute hormonal and metabolic effects to both the GI and GL in healthy individuals at rest using nutrition bars containing a mixture of macronutrients. It was hypothesized that compared with both HGI/ MGL and MGI/ HGL ingestion of the LGI/ LGL test meal would induce a smaller postprandial glycemic response, and a decreased insulin/ glucagon ratio. The major finding of this study was that when different mixed meals were consumed the LGI/ LGL meal resulted in a lower insulin/ glucagon ratio compared to the MGI/ HGL test meal, partially supporting the latter hypothesis.

All test meals irrespective of GI or GL resulted in a serum glucose peak after 15 minutes. While the GL better corresponded to the rank order of the mean $AUC_{glycemic}$ responses of the test meals, neither the GI nor GL explained all the variation in glycemic responses observed. $AUC_{glycemic}$ were 42.16 54.70 and 58.22 $mmol \cdot l \cdot^{-1} min^{-1}$ for the LGI/ LGL, HGI/ MGL and MGI/ HGL test meals, respectively. This compares favorably to data reported by Wolever et al of 66 $mmol \cdot l \cdot^{-1} min^{-1}$ for white bread (HGI) and 29 $mmol.l.min^{-1}$ for lentils (LGI) in healthy non-diabetic subjects [2]. Unexpectedly, differences in glycemia did not reach statistical significance between the three trials despite the wide spread of GI's (27-70%) and GL's (22–81g) of the test meals used [2]. This observation is consistent with some [36,12], but not all studies [7,11,24,33,41,42] that have determined glycemic responses to mixed meals based on the GI and GL. The similarity in glycemic responses in the present study may be partly due to the method of blood-letting used. Venous blood from an anticubital vein rather than capillary blood, typically diminishes the differences in glycemic responses between test foods, which has been

attributed to the arterialization of capillary blood (as used in the GI protocol) and the possible extraction of glucose from venous blood by forearm tissues [2,4,43-45].

Serum insulin responses were significantly lower in the LGI/ LGL compared to the HGI/MGL and MGI/HGL trials. This finding appears indicative of a lower flux of exogenous nutrients from the GI tract and would corroborate the postulated slower rate of digestion and absorption of LGI/ LGL foodstuff [46]. The GL once again better corresponded to the rank order of the mean $AUC_{insulinemic}$ responses to the test meals. Both the GI and GL, however, accounted for comparable portions of variability in postprandial insulinemia ($r = 0.53$, $r = 0.54$, respectively, $p < 0.05$). This finding suggests that when mixed macronutrient food-sources containing significant quantities of CHO, protein and fat (nutrition bars) are consumed both the GI and GL are equally effective predictors of subsequent insulinemia. Previous studies that have determined the glycemic responses to mixed meals have supported the GI's predictive utility in healthy [17,18,20,23] but not diabetic individuals [12,21,36,,47]. The observations of Galgani et al who observed a similarly close relationship between the GL and $AUC_{insulinemic}$ ($r = 0.60$, $p < 0.05$) also appear to corroborate these findings [24]. The above finding however, contrasts to those of Wolever et al who suggested that the GL ($r^2 = 0.90$ $p < 0.05$) is a better predictor of the acute insulinemic demand of mixed meals [25]. The present study demonstrates that protein and fat are less important mediators of postprandial insulinemia ($r = -0.41$, $r = -0.43$ respectively, $p < 0.05$) compared to the CHO content of a meal ($r = 0.339$, $p < 0.05$) [25]. The inverse relationship between the non-CHO content and insulinemic iAUC is likely due to the fact that when the protein and fat amount was lower, the CHO quantity consumed was conversely higher, given the isocaloric nature of the test meals (Table

3.1). Glucose is the principal regulator of pancreatic β cell secretion, while amino acids and fatty acids insulinotropic effects are largely indirect via the amplification of glucose-stimulated insulin secretion [48-52]. Mean $iAUC_{insulinemic}$ in the present study was 2145, 3338 and 3623 $uU.ml.min^{-1}$ for the LGI/LGL, HGI/MGL and MGI/ HGL test meals, respectively. This is significantly lower than 6260, 8020 and 10270 $uU \cdot ml^{\cdot -1} min^{-1}$ for LGI, MGI and HGI respectively reported by Laine et al for similar composite meals [36]. It is unclear why the differences in insulinemia between the two studies was so pronounced, but may also be due to differences in insulin sensitivity of the subjects in the present study compared to other research [53-56].

Serum NEFA concentrations were progressively suppressed following all test meals, but to a greater extent in the MGI/ HGL compared to the other two trials. Similar observations were also reported by Galgani et al [24]. Intuitively, given the hyperinsulinemia also observed in the MGI/ HGL trial, the lower NEFA availability is likely due to a greater insulin-mediated suppression of lipolysis on insulin-sensitive tissue [57]. This postulation was supported by the findings of Wee et al [20,23] who reported elevated FFA & glycerol concentrations following the ingestion of LGI/ LGL compared to HGI/ HGL meals. It may therefore be inferred that the GL is a better indicator of the insulinogenic effects of mixed meals, at least in the extreme (LGL versus HGL). Randle's glucose-fatty acid cycle suggests that increased levels of glucose, inhibit lipid uptake, lipolysis and oxidation [57-60]. It is likely that a greater flux of exogenous CHO across the gut in the MGI/ HGL trial more potently favored glucose metabolism, evidenced by the lower NEFA concentrations observed. Conversely, a lower rate of absorption of exogenous CHO in the LGI/ LGL trial, likely resulted in a lesser glucose mediated

suppression of NEFA availability explaining the higher concentrations observed in this trial [60-64]. Unexpectedly, when NEFA responses were normalized to fasting concentrations, levels were greater in the HGI/ MGL compared to the LGI/ LGL trial, which is indicative of a greater reduction in insulin sensitivity given the concomitant hyperinsulinemia in the former [60,61,64]. This observation in the present study was likely the result of a faster rate of digestion and absorption of exogenous NEFA's from the gut into the systemic circulation from the HGI/ MGL food-source, given the identical fat content between the two trials. Nonetheless, further investigation appears warranted.

Glucagonemia appeared to be greater during the LGI/ LGL compared to the MGI/ HGL trial. The GL once again better corresponded to the rank order of the mean $AUC_{glucagonemic}$ responses to the test meals, although neither the GI nor GL explained the variation in glucagonemia observed. The primary stimuli for glucagon secretion from α-cells are augmented autonomic neural stimulation and circulating AA, along with attenuated concentrations of inhibitory paracrines (insulin and somatostatin) and hypoglycemia [65-69]. Reduced insulin-mediated inhibition of glucagon secretion at the α cells and augmented concentrations of AA likely facilitated the elevated glucagonemia observed following the LGI/ LGL test meal, evidenced by the lower insulinemia in this trial, and a significant relationship between the protein content and $AUC_{glucagonemia}$ ($r = 0.40$, $p = 0.05$) [70]. Hyperglucagonemia during the early postprandial period (~2 hours) has been observed by some [22,23], but not all studies [24] following the consumption of LGI foods. Glucagon acts as chief antagonistic to insulin in maintaining euglycemia by stimulating hepatic glucogenic processes and the uptake of gluconeogenic precursors, whilst suppressing hepatic glycolytic and glycogenic pathways [65,68,70,71]. The heightened

glucagonemia observed following the LGI/ LGL meal suggests that postprandial euglycemia was maintained through a combination of both the continued absorption of exogenous nutrients across from the gastrointestinal tract and hepatic glucose production contributing to splanchnic glucose output.

The insulin/ glucagon molar ratio was also greater following the MGI/ HGL test meal compared to the other two trials, which appears to corroborate the previous observations of Wee et al [23]. This finding suggests that the GL, not the GI may better predict postprandial challenges to glucoregulatory mechanisms evidenced by changes in the underlying hormonal milieu. A higher insulin/ glucagon ratio potently challenges glucose homeostatic mechanisms, by exaggerating the stimulation of anabolic (glycogenensis and lipogenesis) and the suppression of catabolic processes (glycogenolysis, gluconeogenesis and lipolysis) during the early to mid postprandial period [46,56]. In the long-term Ludwig [46] hypothesized that the habitual consumption of HGI (or HGL) meals may initiate a cycle of hyperinsulinemia and insulin resistance that increases β cell demand which may ultimately compromise their function and result in the etiology of type 2 diabetes [46,72,73].

The reliability of the glucagon data also necessitates discussion. Radioimmunological determination was performed in two batches, once for subjects A-F and a second time for subjects G-L (Coefficients of variation (CV) were 7.16%, compared to 29.92%, respectively). While the majority of glucagon concentrations were within the middle 50th percentiles of the standard curve for the first batch, most glucagon responses for the second batch were clustered in the lowest 20th percentiles, with a significant number below the curves detectable limits (< 92.5% bound/ 0.73 pmol). It was

difficult to determine the cause of this inter-batch variability. Statistical analyses still determined differences in the glucagon responses (n=9) between the three trials. Given the differential glucagon responses observed after the first batch of analyses (n = 6), it seems most likely that questionable data was obtained. This may have resulted in a more tepid estimate of glucagonemia during the three trials, and if anything increased the probability of a type 2 not 1 error.

A major novelty of this study was the use of nutrition bars providing a balanced mixture of macronutrients with validated GI and GL values. It was suggested that this would negate some criticisms and limitations of previous studies [12,29,30,32-36,48] that have investigated the GI's predictive capability for mixed meals, by calculating a meal GI (MGI) based on the GI values of the constituent single foods [11]. In conclusion, the present study demonstrates that HGL meals result in an exaggerated insulin/ glucagon ratio and attenuated serum NEFA availability during the early postprandial period, compared to LGL and MGL meals. The GI and GL appear to be equally capable predictors of the acute insulinemic in response to mixed macronutrient meals, but the GL better corresponds to the overall early postprandial insulinogenic effects. Despite these differences, serum glucose was maintained similarly between the three trials.

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Table 3.1: Nutritional intake for the three test meals.

	LGI/ LGL (1g CHO/ kg)	HGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
Volume (g)	206.96 ± 13.76	197.12 ± 13.12	237.03 ± 15.77
Total Carbohydrate (g)	95.20 ± 6.33	94.62 ± 6.30	150.50 ± 10.01
Dietary Fibre (g)	12.42 ± 0.83	11.83 ± 0.79	15.05 ± 1.00
Sugars (g)	62.09 ± 4.13	74.90 ± 4.99	131.68 ± 8.76
Total Useful Carbohydrate (g)	82.78 ± 5.50	82.79 ± 5.51	135.45 ± 9.01
Protein (g)	53.81 ± 3.58	51.25 ± 3.41	31.23 ± 2.08
Total Fat (g)	33.11 ± 2.20	31.54 ± 2.10	16.93 ± 1.13
Saturated Fat (g)	12.42 ± 0.83	11.83 ± 0.79	3.01 ± 0.20
Calories (Kcal)	827.83 ± 55.02	788.47 ± 52.48	827.73 ± 55.08
GI (%)	27*	70**	60**
GL (g)	22.35 ± 1.49	57.95 ± 3.89	81.27 ± 5.41

Values are means ± S.D. * = Clinically validated GI; ** = Estimated GI

Table 3.2. Changes in total serum responses over 150 minutes to the three test meals.

	Time (min)	0 (fasting)	15	30	45	60	90	120	150
Glucose (mmol/l)									
n = 12	LGI/ LGL (1g CHO/ kg)	4.59 ± 0.34	5.56 ± 0.93	5.31 ± 0.81	4.10 ± 0.90	3.91 ± 0.95	4.14 ± 0.86	4.70 ± 0.79	4.68 ± 0.81
	HGI/ MGL (1g CHO/ kg)	4.56 ± 0.47	5.31 ± 0.72	5.02 ± 1.39	4.54 ± 1.56	3.94 ± 1.24	4.11 ± 1.21	4.49 ± 1.40	4.33 ± 0.90
	MGI/ HGL (isocaloric)	4.82 ± 0.60	6.29 ± 0.90	6.16 ± 1.31	4.73 ± 1.25	4.32 ± 1.04	4.36 ± 1.03	4.37 ± 1.27	4.29 ± 1.16
***NEFA (mmol/l)**									
n = 12	LGI/ LGL (1g CHO/ kg)	0.51 ± 0.23 ^{bcdefgh}	0.36 ± 0.15 ^{cdefg}	0.27 ± 0.12	0.23 ± 0.12	0.22 ± 0.09	0.26 ± 0.09	0.26 ± 0.06	0.30 ± 0.07
	HGI/ MGL (1g CHO/ kg)	0.41 ± 0.21 ^{cdefgh}	0.33 ± 0.13 ^{cde}	0.23 ± 0.11	0.22 ± 0.09	0.22 ± 0.09	0.25 ± 0.10	0.28 ± 0.07	0.29 ± 0.07
	MGI/ HGL (isocaloric)	0.48 ± 0.20 ^{bcdefgh}	0.33 ± 0.07 ^{cdefgh}	0.23 ± 0.08	0.18 ± 0.05	0.16 ± 0.04	0.17 ± 0.04	0.17 ± 0.03	0.18 ± 0.05
Insulin (uU/ml)									
n = 12	LGI/ LGL (1g CHO/ kg)	6.99 ± 2.40	30.20 ± 11.43	39.43 ± 15.25	27.71 ± 14.15	21.29 ± 8.73	19.89 ± 7.92	17.58 ± 7.93	15.92 ± 9.20
	HGI/ MGL (1g CHO/ kg)	6.78 ± 2.02	42.02 ± 15.63	45.12 ± 19.18	36.57 ± 16.25	33.71 ± 14.04	27.54 ± 15.93	20.44 ± 12.85	16.75 ± 7.83
	MGI/ HGL (isocaloric)	6.59 ± 1.69	42.68 ± 18.76	47.81 ± 15.63	42.08 ± 16.56	34.13 ± 11.62	27.42 ± 8.13	24.23 ± 18.24	17.11 ± 10.50
Glucagon (pmol/l)									
n = 9	LGI/ LGL (1g CHO/ kg)	13.17 ± 6.32	13.79 ± 8.94	16.32 ± 11.23	20.70 ± 14.03	21.27 ± 11.86	20.25 ± 10.57	21.65 ± 10.38	22.23 ± 10.23
	HGI/ MGL (1g CHO/ kg)	15.09 ± 7.12	14.45 ± 7.68	16.34 ± 8.33	17.27 ± 8.48	19.39 ± 9.06	19.60 ± 10.47	20.18 ± 10.77	19.60 ± 13.31
	MGI/ HGL (isocaloric)	14.19 ± 9.15	13.39 ± 9.30	17.59 ± 8.85	15.40 ± 10.60	22.32 ± 11.25	18.49 ± 9.93	18.10 ± 11.67	16.63 ± 12.43
***Insulin/ glucagon (molar ratio)**									
n = 9	LGI/ LGL (1g CHO/ kg)	5.57 ± 4.42	27.25 ± 24.24	28.51 ± 20.08	16.23 ± 13.91	11.13 ± 8.79	11.06 ± 10.13	7.33 ± 5.44	6.91 ± 4.78
	HGI/ MGL (1g CHO/ kg)	4.94 ± 5.25	29.38 ± 23.89	29.19 ± 23.94	21.73 ± 18.38	15.83 ± 12.70	15.68 ± 16.77	10.07 ± 7.72	10.96 ± 9.29
	MGI/ HGL (isocaloric)	5.60 ± 5.85	56.92 ± 60.14 ^{afgh}	24.28 ± 18.20 ^{afgh}	34.39 ± 31.05 ^{afgh}	14.49 ± 12.35	13.93 ± 8.04	14.52 ± 11.53	12.32 ± 10.57

Values are means ± S.D. a = significantly different from 0 min; b = significantly different from 15 min; c = significantly different from 30 min; d = significantly different from 45 min; e = significantly different from 60 min; f = significantly different from 90 min; g = significantly different from 120 min; h = significantly different from 150 min. * = Interaction effect. $P < 0.05$.

Table 3.3. Changes in incremental serum responses over 150 minutes to the three test meals.

	Time (min)	15	30	45	60	90	120	150
***Glucose (mmol/l)**								
n = 12	LGI/ LGL (1g CHO/ kg)	0.89 ± 0.71^{def}	0.62 ± 0.78^{e}	-0.54 ± 0.88	-0.66 ± 0.91	-0.40 ± 0.76	0.14 ± 0.70	0.13 ± 0.60
	HGI/ MGL (1g CHO/ kg)	0.75 ± 0.85^{ef}	0.46 ± 1.24	-0.02 ± 1.36	-0.62 ± 1.10	-0.45 ± 1.14	-0.07 ± 1.27	-0.23 ± 0.64
	MGI/ HGL (isocaloric)	1.47 ± 0.95^{defgh}	1.34 ± 1.02^{defgh}	-0.09 ± 1.12	-0.50 ± 0.91	-0.46 ± 0.81	-0.45 ± 1.21	-0.53 ± 0.81
***NEFA (mmol/l)**								
n = 12	LGI/ LGL (1g CHO/ kg)	-0.14 ± 0.15^{cdefg}	-0.22 ± 0.21	-0.26 ± 0.21	-0.27 ± 0.21	-0.24 ± 0.22	-0.24 ± 0.23	-0.19 ± 0.23^{de}
	HGI/ MGL (1g CHO/ kg)	-0.08 ± 0.14^{cdef}	-0.18 ± 0.20	-0.19 ± 0.20	-0.19 ± 0.21	-0.16 ± 0.24	-0.13 ± 0.18^{d}	-0.12 ± 0.18^{de}
	MGI/ HGL (isocaloric)	-0.15 ± 0.16^{cdefgh}	-0.25 ± 0.21^{efg}	-0.30 ± 0.21	-0.32 ± 0.22	-0.31 ± 0.22	-0.31 ± 0.22	-0.30 ± 0.22
Insulin (uU/ml)								
n = 12	LGI/ LGL (1g CHO/ kg)	22.39 ± 11.18	33.07 ± 13.50	18.22 ± 10.14	13.10 ± 7.70	11.31 ± 6.87	9.56 ± 5.96	7.96 ± 7.40
	HGI/ MGL (1g CHO/ kg)	35.24 ± 15.89	38.24 ± 19.33	29.79 ± 15.42	26.93 ± 14.31	20.76 ± 15.60	13.66 ± 11.82	9.97 ± 6.74
	MGI/ HGL (isocaloric)	36.08 ± 18.21	41.21 ± 15.17	35.48 ± 16.19	27.54 ± 11.10	20.83 ± 7.65	17.64 ± 18.56	10.52 ± 10.22
Glucagon (pmol/l)								
n = 9	LGI/ LGL (1g CHO/ kg)	2.93 ± 4.36	5.41 ± 6.11	9.20 ± 9.23	10.13 ± 7.01	9.10 ± 6.50	8.89 ± 6.01	9.59 ± 5.61
	HGI/ MGL (1g CHO/ kg)	3.13 ± 4.02	5.02 ± 3.59	7.07 ± 2.76	7.05 ± 6.01	8.28 ± 7.13	9.98 ± 3.73	8.28 ± 10.32
	MGI/ HGL (isocaloric)	2.04 ± 4.63	3.80 ± 4.41	5.29 ± 4.48	9.00 ± 6.69	8.37 ± 4.94	8.64 ± 8.54	7.17 ± 6.23
***Insulin/ glucagon (molar ratio)**								
n = 9	LGI/ LGL (1g CHO/ kg)	23.79 ± 25.37	26.62 ± 21.14	12.51 ± 12.25	7.22 ± 7.62	6.83 ± 7.78	4.16 ± 6.19	4.54 ± 9.73
	HGI/ MGL (1g CHO/ kg)	25.67 ± 24.42	25.48 ± 21.38	17.84 ± 17.83	11.78 ± 9.66	11.97 ± 16.68	6.18 ± 7.37	7.25 ± 11.04
	MGI/ HGL (isocaloric)	52.45 ± 58.27^{fgh}	19.66 ± 19.43^{fgh}	32.05 ± 31.87^{fgh}	12.33 ± 13.61	11.59 ± 8.63	10.79 ± 9.43	8.59 ± 10.97

Values are means ± S.D. b = significantly different from 15 min; c = significantly different from 30 min; d = significantly different from 45 min; e = significantly different from 60 min; f = significantly different from 90 min; g = significantly different from 120 min; h = significantly different from 150 min. * = Interaction effect. $P < 0.05$.

Table 3.4. Total areas under the curve (tAUC) for glucose, NEFA, insulin, glucagon and insulin/ glucagon ratio responses to the three test meals.

	LGI/ LGL (1g CHO/ kg)	HGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
Glucose ($mmol \cdot l^{-1} min^{-1}$)	682.38 ± 80.51	668.78 ± 139.49	717.48 ± 114.25
***NEFA ($mmol \cdot l^{-1} min^{-1}$)**	41.56 ± 13.03^{k}	39.96 ± 11.68^{k}	31.14 ± 4.98
***Insulin ($uU \cdot ml^{-1} min^{-1}$)**	3354.23 ± 1082.28^{jk}	4355.40 ± 1333.89	4612.28 ± 878.81
***Glucagon ($pmol \cdot l^{-1} min^{-1}$)**	3425.17 ± 1383.93^{k}	3150.79 ± 1307.20	2752.75 ± 1621.10
Insulin/ glucagon ($mol \cdot l^{-1} min^{-1}$)	1490.19 ± 1059.04	2088.16 ± 1798.60	4320.15 ± 4872.10

Values are means ± S.D. j = significantly different from HGI/ MGL (1g CHO/ kg); k = significantly different from MGI/ HGL (isocaloric). * = Test meal main effect. $P < 0.05$.

Table 3.5. Incremental areas under the curve (iAUC) for glucose, NEFA, insulin, glucagon and insulin/ glucagon ratio responses to the three test meals.

	LGI/ LGL (1g CHO/ kg)	HGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
Glucose ($mmol \cdot l^{-1} min^{-1}$)	42.16 ± 32.70	54.70 ± 53.90	58.22 ± 33.22
NEFA ($mmol \cdot l^{-1} min^{-1}$)	-	-	-
***Insulin ($uU \cdot ml^{-1} min^{-1}$)**	2145.27 ± 741.91^{jk}	3338.49 ± 1255.68	3623.13 ± 794.79
***Glucagon ($pmol \cdot l^{-1} min^{-1}$)**	1342.20 ± 799.83^{k}	1022.68 ± 628.94	873.36 ± 724.21
Insulin/ glucagon ($mol \cdot l^{-1} min^{-1}$)	674.31 ± 446.28	1327.66 ± 1351.87	3248.13 ± 3942.14

Values are means ± S.D. j = significantly different from HGI/ MGL (1g CHO/ kg); k = significantly different from MGI/ HGL (isocaloric). * = Test meal main effect. $P < 0.05$.

Table 3.6: Correlations between incremental areas under the curve (iAUC) and the GI, GL, available CHO, protein and fat.

	GI (g)	GL (g)	CHO (g)	Protein (g)	Fat (g)
Glucose iAUC ($mmol \cdot l^{-1} min^{-1}$)	r = 0.199 p = 0.251	r = 0.132 p = 0.487	r = 0.109 p = 0.534	r = 0.050 p = 0.777	r = 0.065 p = 0.712
NEFA iAUC ($mmol \cdot l^{-1} min^{-1}$)	-	-	-	-	-
Insulin iAUC ($uU \cdot ml^{-1} min^{-1}$)	r = 0.525 p = 0.001*	r = 0.544 p = 0.000*	r = 0.339 p = 0.043*	r = -0.408 p = 0.014*	r = -0.427 p = 0.009*
Glucagon iAUC ($pmol \cdot l^{-1} min^{-1}$)	r = 0.108 p = 0.571	r = 0.132 p = 0.487	r = 0.087 p = 0.647	r = 0.405 p = 0.027*	r = 0.296 p = 0.112
Insulin/ glucagon ratio iAUC ($mol \cdot l^{-1} min^{-1}$)	r = 0.247 p = 0.224	r = 0.328 p = 0.102	r = 0.170 p = 0.278	r = 0.485 p = 0.010*	r = 0.467 p = 0.016*

Values are means ± S.D. * = $p < 0.05$.

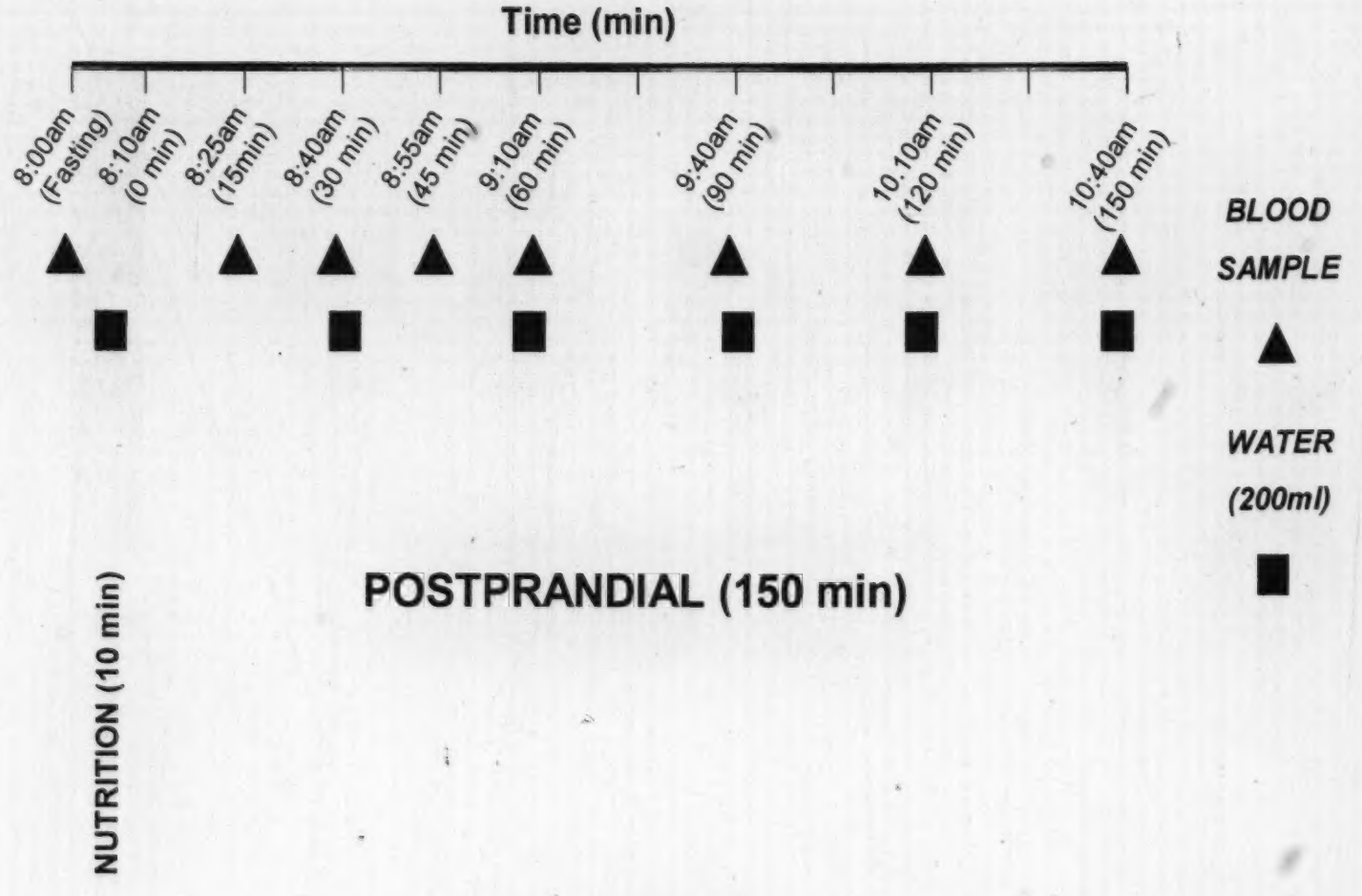

Figure 3.1: Trial procedure

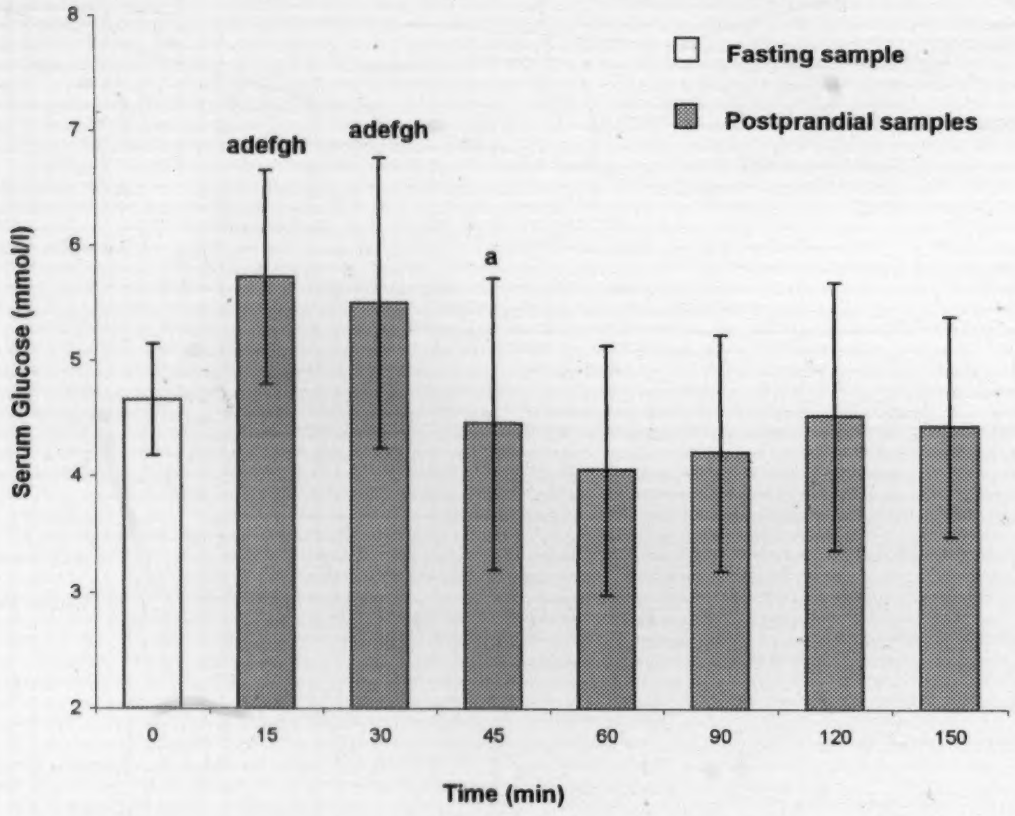

Figure 3.2: Main effect of time collapsed across the three test meals on the total glucose response over 150 minutes. Values are means ± S.D. a = significantly different from 0 min; d = significantly different from 45 min; e = significantly different from 60 min; f = significantly different from 90 min; g = significantly different from 120 min; h = significantly different from 150 min. Time main effect. $P < 0.05$.

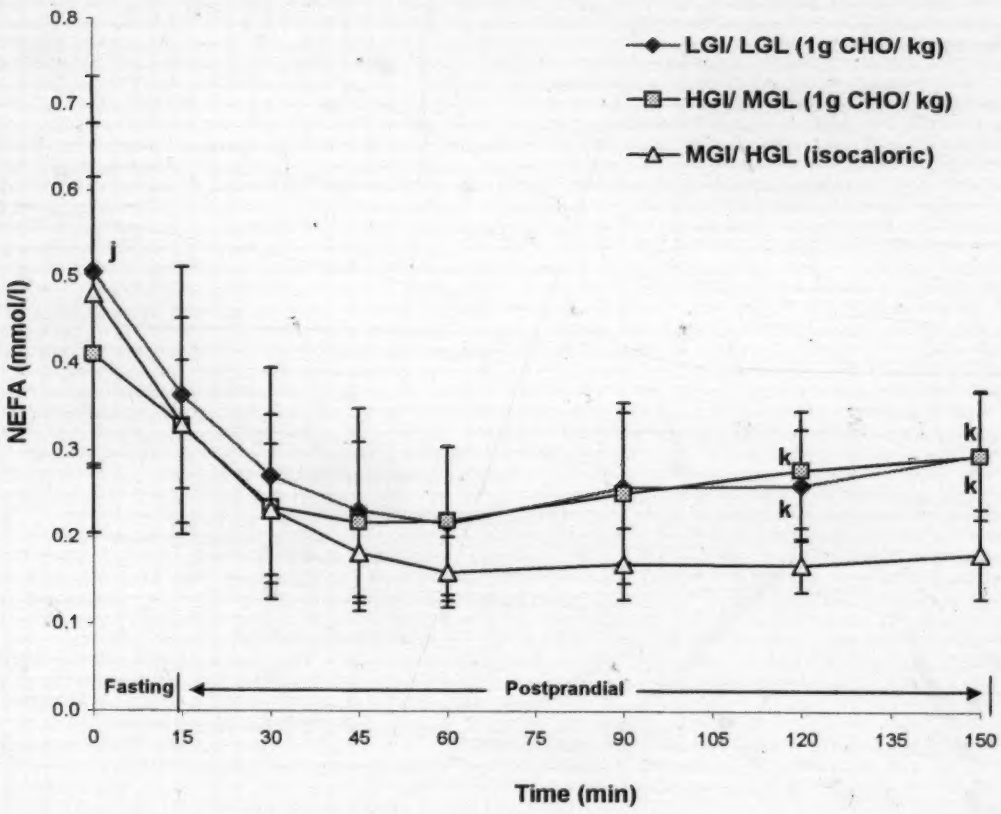

Figure 3.3: Changes in the total NEFA response over 150 minutes between the three test meals. Values are means ± S.D. j = significantly different from HGI/ MGL (1g CHO/ kg); k = significantly different from MGI/ HGL (isocaloric). Interaction effect. $P < 0.05$.

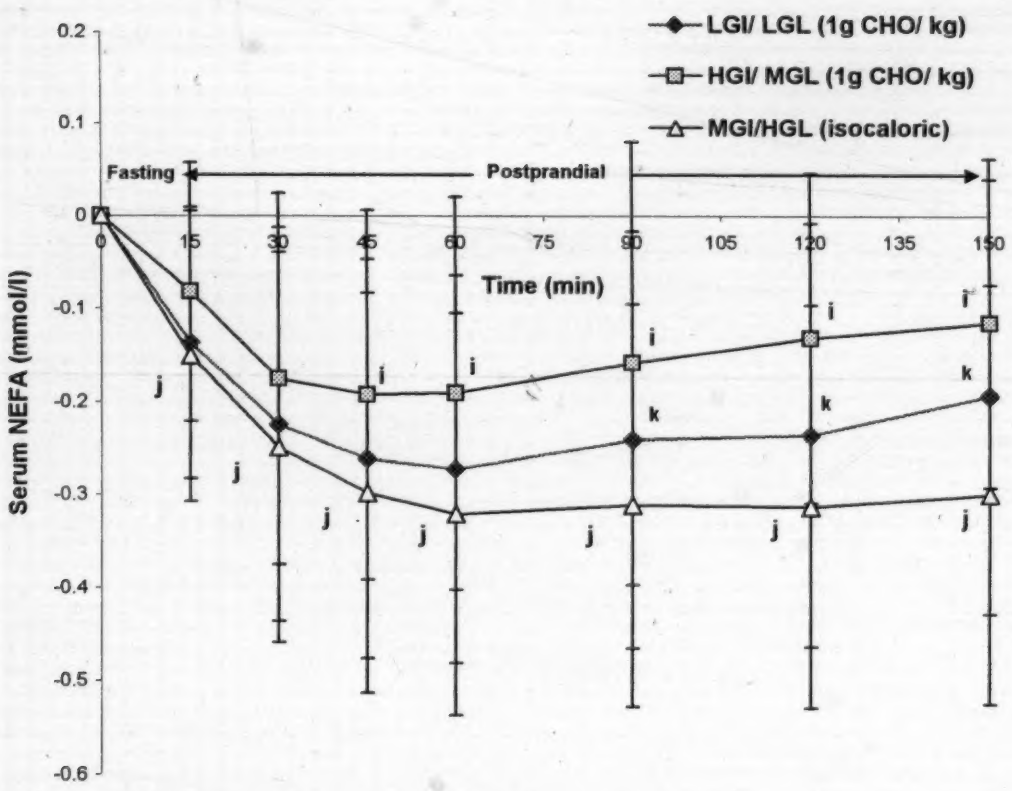

Figure 3.4: Changes in the incremental NEFA response over 150 minutes between the three test meals. Values are means ± S.D. i = significantly different from LGI/ LGL (1g CHO/ kg); j = significantly different from HGI/ MGL (1g CHO/ kg); k = significantly different from MGI/ HGL (isocaloric). Interaction effect. $P < 0.05$.

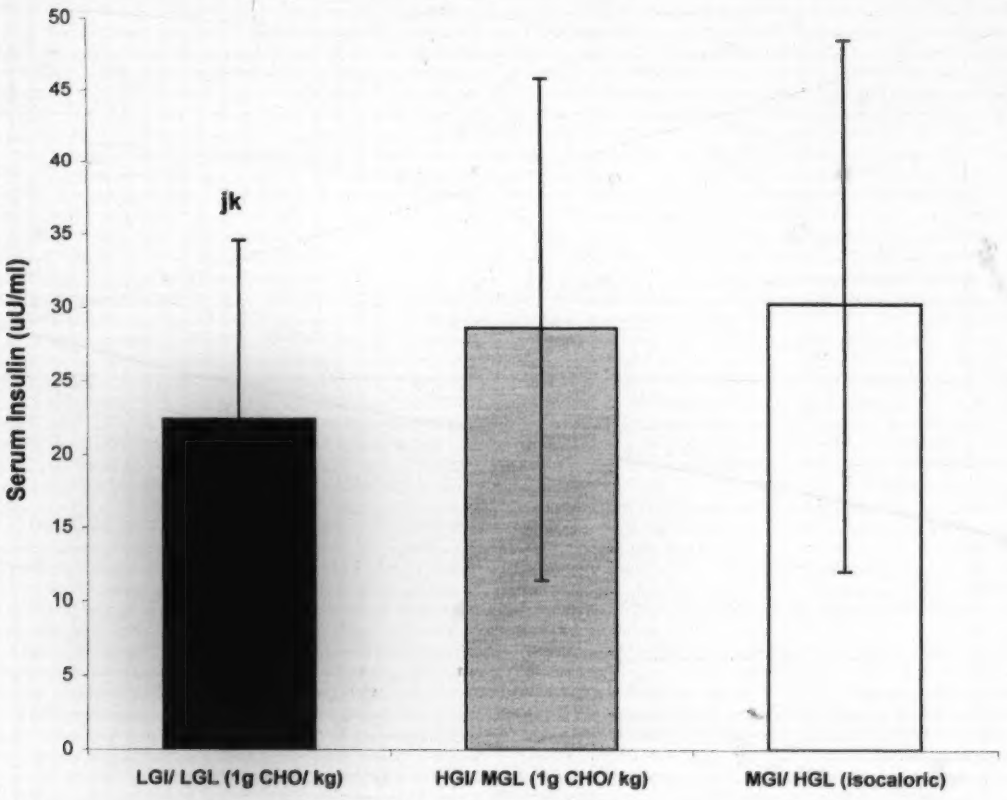

Figure 3.5: Main effect of test meal collapsed across all time points on the total insulin response. Values are means ± S.D. j = significantly different from HGI/ MGL (1g CHO/ kg); k = significantly different from MGI/ HGL (isocaloric). Test meal main effect. $P < 0.05$.

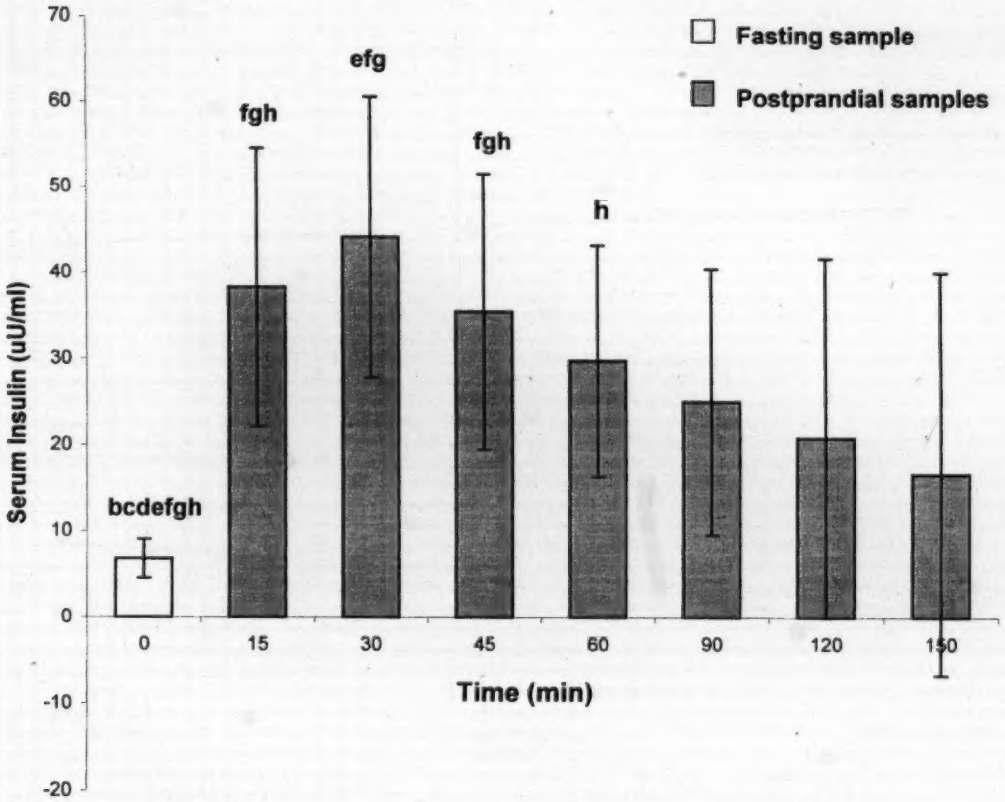

Figure 3.6: Main effect of time collapsed across the three test meals on the total insulin response over 150 minutes. Values are means ± S.D. b = significantly different from 15 min; c = significantly different from 30 min; d = significantly different from 45 min; e = significantly different from 60 min; f = significantly different from 90 min; g = significantly different from 120 min; h = significantly different from 150 min. Time main effect. $P < 0.05$.

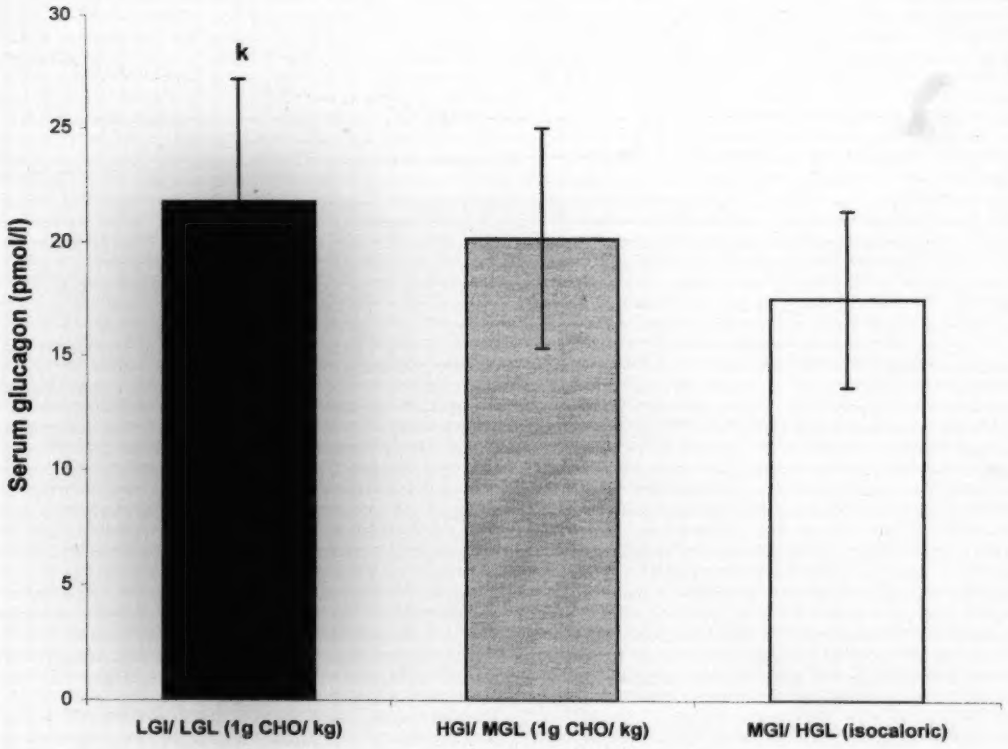

Figure 3.7: Main effect of test meal collapsed across all time points on the total glucagon response. Values are means ± S.D. k = significantly different from MGI/ HGL (isocaloric). Trial main effect. $P < 0.05$.

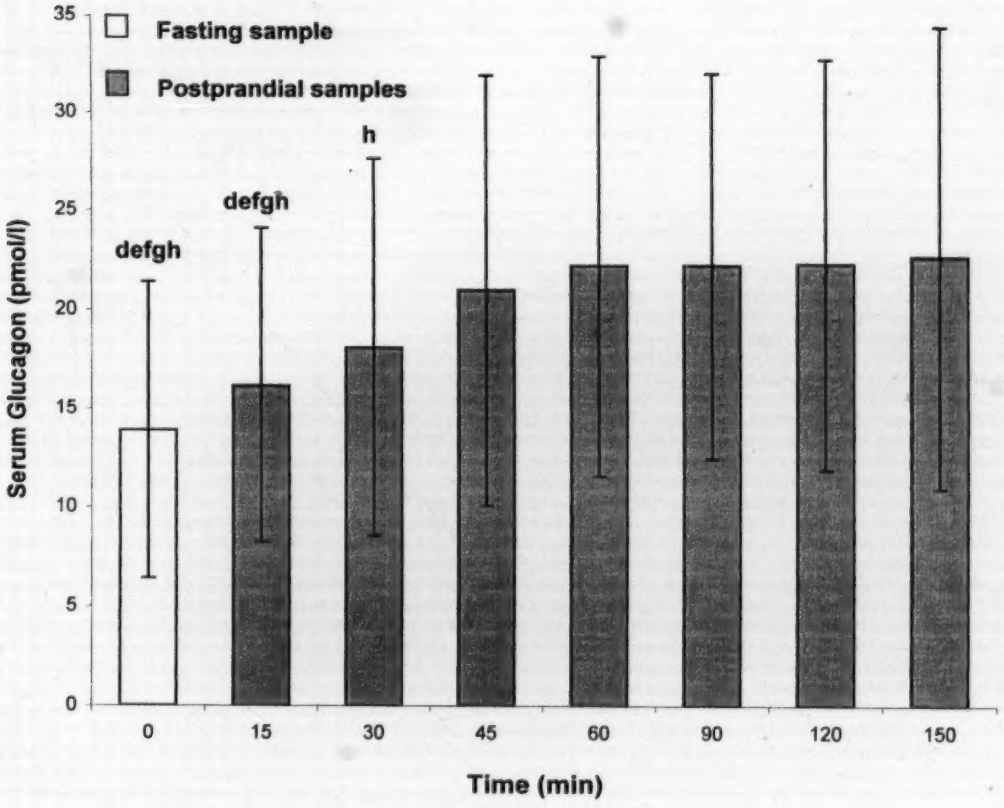

Figure 3.8: Main effect of time collapsed across the three test meals on the total glucagon response over 150 minutes. Values are means ± S.D. d = significantly different from 45 min; e = significantly different from 60 min; f = significantly different from 90 min; g = significantly different from 120 min; h = significantly different from 150 min. Time main effect. $P < 0.05$.

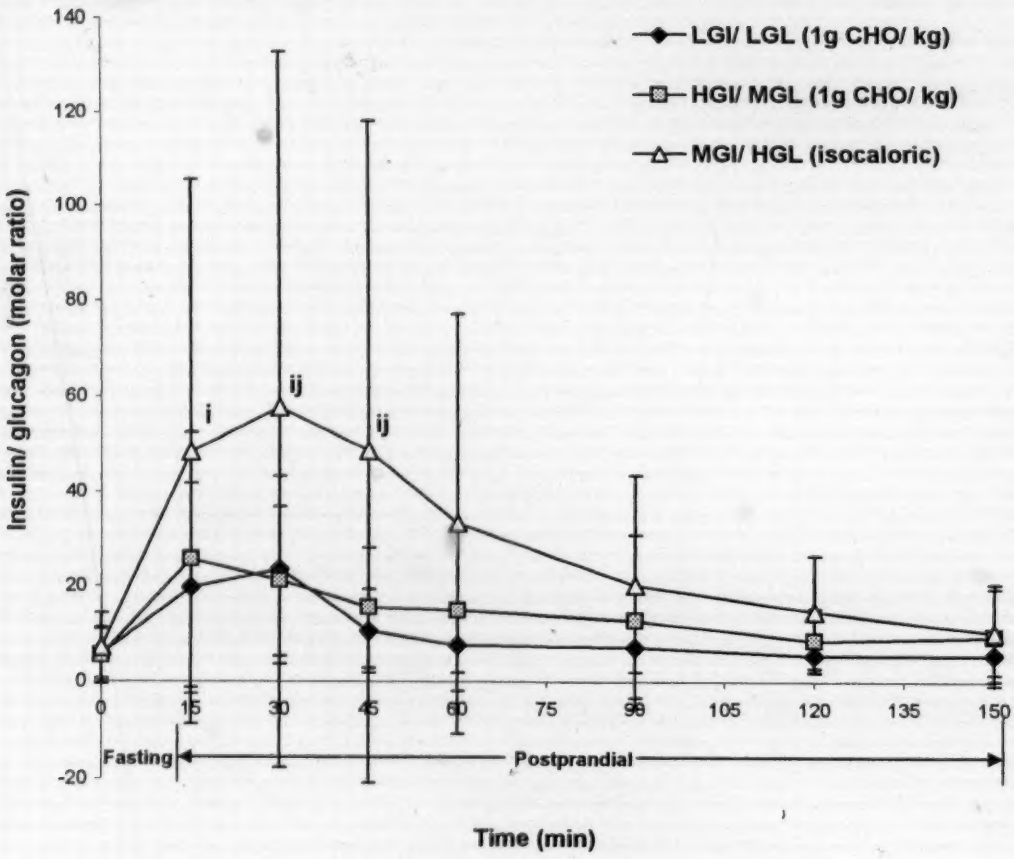

Figure 3.9: Changes in the total insulin/ glucagon response over 150 minutes between the three test meals. Values are means ± S.D. i = significantly different from LGI/ LGL (1g CHO/ kg); j = significantly different from HGI/ MGL (1g CHO/ kg). Interaction effect. $P < 0.05$.

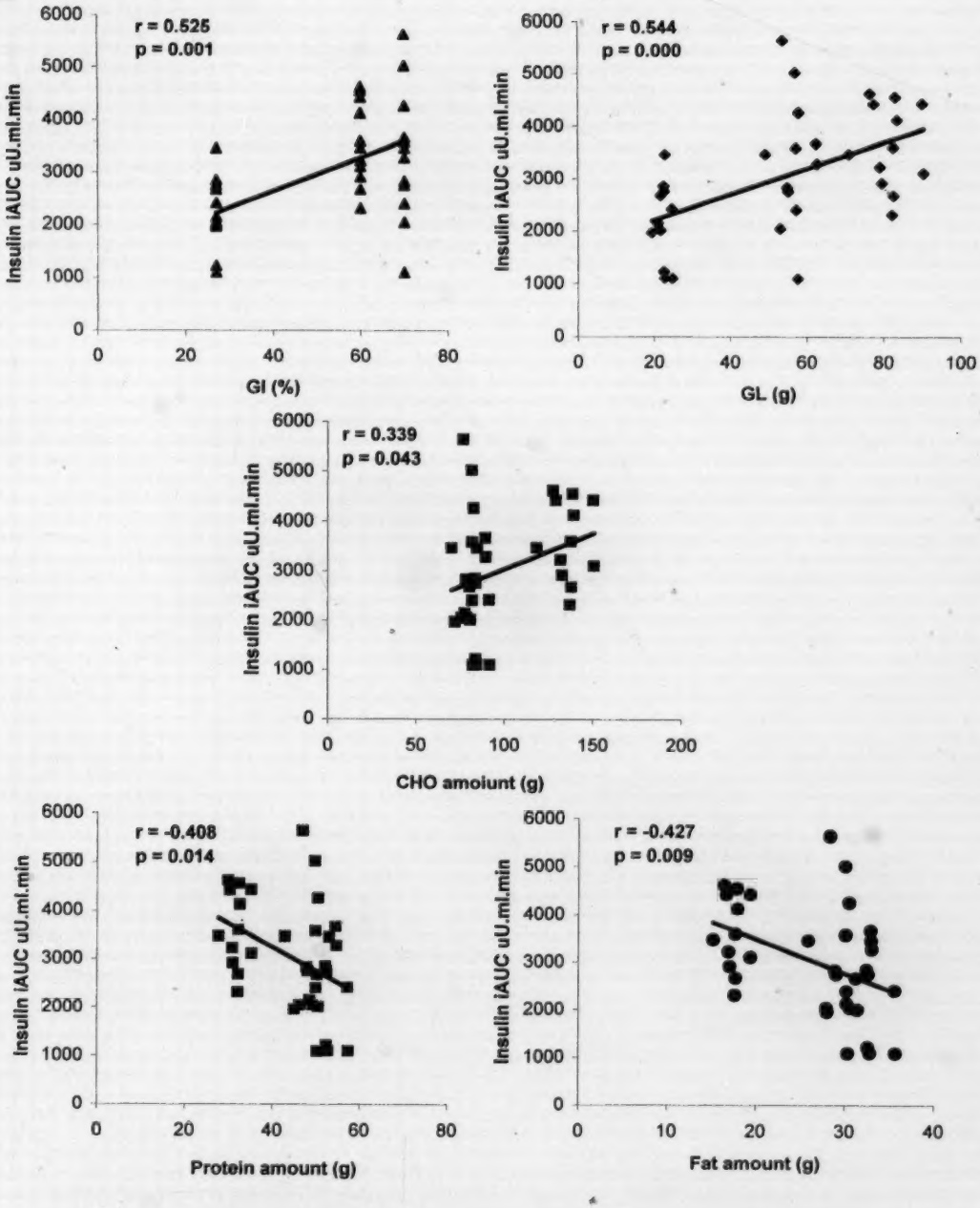

Figure 3.10: Correlations between the incremental areas under the insulinemic responses curve (iAUC) and the fat, protein, available CHO contents; glycemic index (GI), and glycemic load (GL). Values are means ± S.D. $P < 0.05$.

CHAPTER 4 – STUDY TWO

The effect of the glycemic index and load on the glucose and gluco-regulatory hormonal response during exercise.

ABSTRACT

The aim of this study was to examine the effect of the GI and GL on the glucose and gluco-regulatory hormonal responses during prolonged cycling. Five trained males cycled at 10% below the individual ventilatory threshold (VT) for 105 minutes after ingesting a low glycemic index/ load (LGI/ LGL) and moderate glycemic index/ load (MGI/ MGL) nutrition bars, providing 1g CHO per kg body mass, and a moderate glycemic index/ high glycemic load (MGI/ HGL) nutrition bar in an isocaloric amount to the LGI/ LGL trial, 60 minutes pre-exercise. Only during MGI/ HGL (isocaloric) was RER maintained during exercise. All feedings resulted in a blood glucose nadir and insulin peak immediately pre-exercise. Ingestion of LGI/ LGL resulted in greater ($P < 0.05$) insulin responses after 35 and 105 minutes of exercise than MGI/ MGL (1g CHO/ kg). Glucagon concentrations in LGI/ LGL after 35 and 105 minutes of exercise were greater ($P < 0.05$) than both MGI trials. The insulin/ glucagon ratio at 35 minutes and the non-esterified fatty acid (NEFA) concentration after 70 minutes of exercise were greater ($P < 0.05$) in LGI/ LGL than MGI/ MGL (1g CHO/ kg). Consumption of LGI/ LGL feedings 60 minutes pre-exercise result in hyperglucagonemia during exercise compared to MGI/ MGL/ HGL feedings.

Additional information pertaining to study one is in appendices 1, 3, 5, 8, 9 and 10.

INTRODUCTION

Carbohydrate (CHO) feedings before and during prolonged exercise are essential for optimal endurance performance by maintaining CHO availability to the exercising musculature and central nervous system (CNS) [1-3]. Exogenous CHO is beneficial in three ways: (i) stimulating muscle [4,5] and (ii) hepatic glycogenesis [6,7] prior to exercise (pre-exercise meals) and (iii) by providing an ancillary source of splanchnic glucose output to maintain euglycemia via continued absorption from the gastrointestinal space, during exercise (feedings the hour before and during exercise) [8,9]. A potential disadvantage of pre-exercise CHO intake is transient hypoglycaemia at exercise onset due to a negative imbalance between insulin and contraction mediated peripheral glucose uptake and hepatic glucose production (HGP) [8,10]. Such a metabolic effect is most pronounced when CHO is consumed the hour before exercise. The efficacy of consuming CHO during this period is controversial due to conflicting evidence regarding performance benefits. This nutritional strategy has been shown to increase [11-15], have no effect [16-20] or decrease [21,22] endurance performance.

The glycemic index (GI), a standardized system of classification for carbohydrate (CHO) foods based on their postprandial blood glucose response and has been advocated as a useful method for manipulating CHO intake in sports nutrition [8,23]. When CHO consumption the hour before exercise is quantified using the GI literature shows that LGI foods elicit smaller postprandial glycemic and insulinemic perturbations compared to HGI foods [18,20,24] and may [10,12,25] or may not improve performance [16-18]. Some authors have advocated the consumption of LGI foods the hour prior to exercise [26], but evidence appears to suggest a lower bioavailability during exercise compared to HGI feedings

[27,28]. Most recently the glycemic load (GL) was introduced [29,30]. GL = (GI*Grams of available CHO)/ 100 [29,30]. Like the GI, the GL could be a useful tool in sports nutrition.

Studies examining the effect of consuming CHO the hour prior to exercise using the GI have frequently used mixed meals [9,12,13,17,18,25,28,31]. The GI's predictive capability for mixed meals based on the calculation of a meal GI (MGI) using values from each of the constituent single foods [32] has been questioned given the myriad of factors that affect postprandial glycemia [33-39]. Some studies have supported the GI's predictive capabilities in this context [32,,40,41], while others have not [42-46]. Nutrition bars are a convenient and readily accessible food-source for active individuals. Given their balanced macronutrient composition and known GI and GL values [47-49] they would appear to facilitate more valid investigation into these indices efficacy in characterizing the metabolic responses to mixed meals consumed the hour prior to exercise.

Few if any studies have characterized the glucoregulatory hormonal and metabolic effects of consuming CHO the hour prior to exercise based on the GI and GL indices, nor with using nutrition bars as the food source. The purpose of this study was to determine the effects of the GI and GL on glucose and gluco-regulatory hormonal responses during 105 minutes of endurance cycling. It was hypothesized that: (i) ingestion of LGI/ LGL compared to HGI/ HGL nutrition 60 minutes prior to exercise would elicit an augmented counterregulatory response during prolonged cycling; (ii) ingestion of LGI/ LGL nutrition would negate the transient hypoglycaemic and hyperinsulinemic perturbations observed following HGI nutrition at exercise onset.

METHODS

Participants: Five endurance trained male cyclists (30.2 ± 6.5 years, 1.8 ± 0.0 m, 77.5 ± 3.5 kg and $VO_{2\ peak}$ of 4.31 ± 0.62 L/min) volunteered as subjects for this study after being informed of the risks associated with participation and completing a letter of informed consent. The study was approved by the Faculty of Physical Education and Recreation Research Ethics Board at the University of Alberta.

Exercise testing and experimental protocol: Laboratory testing necessitated five separate visits. The study was of a within subject design with the experimental trials completed in a randomized order. Prior to commencement of the exercise testing each subject attended the laboratory for an orientation session during which further details of the study were explained, descriptive data was gathered and subjects were familiarized with the testing procedures and equipment. Subjects were asked to record the type and amount of food and beverage consumed in 24 hours during a "typical" day at this time. The 24-hour dietary record was analyzed using a software program (Food Processor II, EHSA, USA) to determine macronutrient content and modified where necessary to establish a percent breakdown of macronutrients of 60% carbohydrate, 15% protein and 25% fat. Subjects were asked to consume identical amounts and types of food specified on their diet record, the day prior to each trial (3609.5 ± 751.7 kcal which consisted of 61 ± 4.9% carbohydrate, 14.4 ± 1.7% protein and 24 ± 3.5% fat).

All exercise trials were conducted on the subjects' own bike on an indoor magnetic training device that allowed resistance to be applied to the rear wheel (Tacx 100, Netherlands) and at a tire pressure and cadence of 120 psi and 90 revolutions per minute (rpm), respectively. Previous pilot work in the laboratory validated the use of the

magnetic device for determining graded and constant intensity exercise responses. Participants' were provided with a training tire (Continental, Germany) designed specifically for use on indoor magnetic cycle trainers. Subjects were asked to abstain from physical activity, alcohol and smoking the day prior to all exercise testing.

Peak oxygen consumption ($VO_{2\ peak}$) and ventilatory threshold (VT) were determined during incremental graded exercise to volitional exhaustion similar to a protocol outlined by the American College of Sports Medicine (ACSM) guidelines for a graded cycle exercise test [50]. Subjects began cycling at 100 Watts, with the power output increased in 30 Watt increments, initially every two minutes until VT was surpassed, and thereafter at one minute intervals until volitional exhaustion. Expired gas was collected continuously during the test and analyzed using a calibrated metabolic measurement system (ParvoMed True Max 2400, USA). Heart rate was recorded every minute using a heart rate monitor (Polar, Finland). Volitional exhaustion was defined as the point at which the subject could not continue to exercise due to fatigue. Peak VO_2 was defined as the highest Vo_2 recorded during the maximal exercise test associated with a respiratory exchange ratio greater than 1.1 and achievement of age predicted (220 – age) or known maximum heart rate. VT was determined using the V-slope method [51]. A five minute warm-up and cool-down along with a stretching period was included.

Subjects reported to the laboratory between 7:00 and 8:00 am on three subsequent occasions, following a 10-hour overnight fast. The 3 trials were separated by at least 5 days, and were conducted in no more than a 4-week period. On arrival in the laboratory an intravenous cathelon was inserted and a fasting blood sample obtained (-80 minutes). Subjects then consumed one of three nutrition bar feedings (single blind randomized

order) within 10 minutes of the fasted sample. The feedings were a low GI/ GL (LGI/ LGL 1g CHO/ kg) nutrition bar (GI = 27, GL = 18.19 ± 0.90) and a moderate GI/ GL (MGI/ MGL 1g CHO/ kg) nutrition bar (GI = 60, GL = 42.29 ± 2.08) both providing 1 gram of carbohydrate per kilogram of body weight. Previous research has demonstrated that this amount carbohydrate can significantly alter glucose control during prolonged sub-maximal exercise [17,18]. Since LGI/ LGL (1g CHO/ kg) provided more total calories compared to MGI/ MGL (1g CHO/ kg), the MGI nutrition bar was consumed on a second occasion, but this time in an isocaloric amount compared to LGI (MGI/ HGL isocaloric) (GI = 60, GL = 67.34 ± 3.31). LGI/ LGL (1g CHO/ kg) and MGI/ HGL (isocaloric) both provided 673.6 ± 33.3 kcal, while MGI/ MGL (1g CHO/ kg) provided 422.9 ± 20.8 kcal. All meals were provided along with ~ 250 ml of water. Ten minutes were permitted to consume the test meals, with the actual time taken recorded and subjects asked to replicate this for all proceeding trials. After 1 hour of rest, a pre-exercise blood sample was taken (-10 minutes). Previous research has demonstrated consuming carbohydrate foods 1 hour prior to exercise can elicit significant changes in glucose control and endurance performance [12]. Subjects' pre-exercise weight was recorded following which subjects warmed up for ten minutes and then cycled for 105 minutes at the heart rate and ventilatory parameters that elicited an intensity equivalent to 10% below the individual VT [52]. Previous studies have demonstrated exercise at 70% $VO_{2\,max}$ for 105 minutes was able to significantly affect glucose control [4,52,53]. During exercise subjects were provided with 250ml of water every 20 minutes [54]. Following completion of 105 minutes of cycling subjects were permitted a 5-minute cooling off and stretching period. The intensities (power output and gear ratio's) of the warm up and cool down were recorded

and the subjects were asked to replicate this for the proceeding trials. Subjects' post-exercise weight was also recorded in order to monitor any fluid loss due to dehydration.

Heart rate was monitored and recorded in the final 30 seconds of 5-minute intervals (Polar Electro Sports Tester PE 3000, Finland) during the 105 minutes of sub-maximal cycling. Expired gas measurements were taken using a calibrated metabolic cart (ParvoMed True Max 2400, USA) for 5-minute periods after 30, 65 and 100 minutes of exercise. Throughout exercise subjects were asked to rate their level of perceived exertion using the Borg scale [55] which ranges from 7-20 (7- extremely easy and 20 being extremely hard), at 15-minute intervals. In addition to the fasting and pre-exercise blood samples (-80 and -10 minutes), further samples were obtained from the intravenous cathelon after 35, 70 and 105 minutes of sub-maximal exercise, along with 60 minutes after exercise cessation (170 minutes). Figure 4.1 depicts the trial procedure.

Analytic techniques: 4 milliliters (ml) of blood were collected at each sampling time, using a 22 gauge cathelon inserted by a registered nurse, and obtained with a syringe. 0.5ml of sterile saline (0.9% NaCl) was used to keep the cathelon patent during each trial. Prior to obtaining the sample, approximately 1 ml of blood was withdrawn and discarded to ensure the subsequent sample was not diluted by saline. Whole blood was analyzed immediately for hematocrit in duplicate following centrifugation for 5 minutes in a 50-ul micro centrifuge tube and blood lactate using a portable lactate analyzer (Lactate-Pro test meter, Germany). The remaining blood was allowed to clot (~40 minutes), centrifuged at 1500 xg, with the serum supernatant drawn off and stored at -80° C for later analysis. Glucose was measured using the glucose oxidase method on a spectrophotometer (Sigma-Aldrich, USA) while non-esterified fatty acids (NEFA) were

determined by a commercially available enzymatic colorimetric technique (Wako Chemicals USA). Insulin, glucagon and cortisol concentrations were determined using commercially available radioimmunoassay (RIA) kits (Diagnostic Products Corporation (DPC), Los Angeles, USA). All analyses were performed in duplicate. In order for the mean of the duplicates to be accepted, the coefficient of variation (CV) had to less than 15% failing which an appropriate singlet was selected that was within two standard deviations (S.D.) of preceding and proceeding values and which also fitted the trend of the data. Mean coefficients of variation (CV) for the RIA's were 14.3, 8.09 and 4.43% for insulin, glucagon and cortisol respectively. The total serum responses were normalized to zero concentration. Incremental serum responses were normalized to fasting concentrations. Total (tAUC) and incremental iAUC) areas under the curves were calculated geometrically using the trapezium rule, with only the area above the normalized concentrations included in the area calculations [32].

Statistical Analyses: Statistica (Statsoft, USA) was used for all data analysis. Data were expressed as means ± S.D. Due to the small sample size, non-parametric, Friedman two-way analysis of variance (ANOVA) was used to determine any significant differences between the 3 trials. Wilcoxon matched paired tests were used to locate differences when the Friedman two-way ANOVA revealed a significant interaction. An alpha of $p < 0.05$ was considered statistically significant.

RESULTS

The mean power output, VO_2, RER, heart rate and carbohydrate oxidation (measured by indirect calorimetry) throughout 105 minutes of sub-maximal exercise

during the three trials were 207.61 ± 30.17 Watts, 3.03 ± 0.41 L/min, 0.93 ± 0.03, 148 ± 11 bpm and 305.47 ± 52.77 grams. None of these values differed when comparing the three trials (data not shown). RER values for the three sampling intervals did differ over time however ($P < 0.03$), with the RER value after 105 and 65 minutes of cycling during LGI/ LGL and MGI/ MGL (1g CHO/ kg) respectively, lower when compared to after 35 minutes of exercise (Fig. 4.2).

No difference was observed in the magnitude of change in hematocrit when comparing the 3 trials ($P > 0.05$). Blood lactate concentration was higher ($P < 0.05$) after 35 minutes of exercise when compared to –10 minutes in all trials, while the lactate concentration after 105 minutes of exercise was lower ($P < 0.05$) in LGI/ LGL compared with MGI/ MGL (1g CHO/ kg).

Ingestion of the meal during LGI/ LGL resulted in a lower ($P < 0.05$) total serum glucose nadir at –10 minutes and a higher ($P < 0.05$) concentration after 70 minutes of exercise compared with MGI/ MGL (1g CHO/ kg) (Fig. 4.3). Serum glucose concentrations at –10 minutes during LGI/ LGL were lower ($P < 0.05$) than all other time points, while the peak concentration at 70 minutes was greater ($P < 0.05$) than 105 and 170 minutes. Serum glucose concentrations during the two MGI trials peaked after 35 minutes of exercise, which was significantly greater ($P < 0.05$) than values at –10 and 105 minutes. Total serum glucose concentrations during the 2 trials at –80 minutes were also significantly greater ($P < 0.05$) when compared to 105 minutes (Tables 4.2 and 4.3).

Ingestion of the MGI/ MGL (1g CHO/ kg) resulted in a greater ($P < 0.05$) peak total serum NEFA concentration at 170 minutes compared with MGI/ HGL (isocaloric) (Fig. 4.4) and a lower ($P < 0.05$) incremental value at 70 minutes than LGI/ LGL (Fig.

4.5). Serum NEFA concentrations at 70, 105 and 170 minutes during all trials were greater ($P < 0.05$) than –10 and 35 minutes. Serum NEFA concentrations at 70 minutes were also greater ($P < 0.05$) than 105 and 170 minutes in the MGI trials and 170 minutes during LGI/ LGL (Tables 4.2 and 4.3).

Ingestion of the MGI/ MGL (1g CHO/ kg) resulted in a lower ($P < 0.05$) total serum insulin concentration at 35 and 170 minutes when compared to MGI/ HGL (isocaloric). Total serum insulin concentration after 105 minutes of exercise and the incremental concentrations at 35 and 70 minutes were lower ($P < 0.05$) in MGI/ MGL (1g CHO/ kg) compared to LGI/ LGL (Fig. 4.6 and 4.7). Serum insulin concentrations peaked at –10 minutes during the trials, which was greater ($P < 0.05$) than all proceeding values. The nadir in serum insulin occurred after 105 minutes of exercise and was lower ($P < 0.05$) than –80, –10, 35 and 70 minutes in all trials. Serum insulin concentrations after 35 minutes of exercise were greater ($P < 0.05$) than 70 and 170 minutes during LGI/ LGL and 70 minutes during MGI/ HGL (isocaloric) (Tables 4.2 and 4.3).

Ingestion of the LGI/ LGL nutrition resulted in greater ($P < 0.05$) serum glucagon concentrations at 35 and 105 minutes compared to MGI/ MGL (1g CHO/ kg) and greater ($P < 0.05$) incremental serum glucagon concentrations at 35, 70 and 105 minutes of exercise than in the MGI/ HGL (isocaloric) trial. Total serum glucagon concentration at –10 minutes and serum glucagon concentrations at 35 minutes in MGI/ MGL (1g CHO/ kg) were also lower compared to MGI/ HGL (isocaloric) (Figs 4.8 and 4.9). Serum glucagon concentrations at 35 minutes were greater ($P < 0.05$) than 170 minutes in LGI/ LGL and MGI/ MGL (1g CHO/ kg) and –10 and 170 minutes in MGI/ HGL (isocaloric). Serum glucagon concentrations after 70 minutes of exercise were also greater ($P < 0.05$)

when compared to –10 and 35 minutes, while the glucagon values at 105 minutes were greater ($P < 0.05$) than 35 and 170 minutes during LGI/ LGL and MGI/ MGI (Tables 4.2 and 4.3).

Ingestion of the LGI/ LGL nutrition resulted in a greater ($P < 0.05$) insulin/ glucagon ratio at 35 minutes when compared to MGI/ MGL (1g CHO/ kg) (Fig 4.10). During all three trials, the peak insulin/ glucagon ratio occurred at –10 minutes, which was greater ($P < 0.05$) than all proceeding time points, while the nadir in the insulin/ glucagon ratio occurred at 105 minutes which was lower ($P < 0.05$) than –10, 35 and 170 minutes. The insulin/ glucagon ratio at 70 minutes was lower ($P < 0.05$) than –10 and 35 minutes during LGI/ LGL and MGI/ MGL (1g CHO/ kg). The total insulin/ glucagon ratio at –80 minutes during all three trials was greater ($P < 0.05$) than –10 and 35 minutes (Tables 4.2 and 4.3).

Ingestion of the LGI/ LGL nutrition resulted in a lower ($P < 0.05$) total cortisol response at 70 minutes compared to MGI/ MGL (1g CHO/ kg) (Fig 4.11). During MGI / MGL (1g CHO/ kg), the serum cortisol concentrations at 35 minutes were lower ($P < 0.05$) than 70, and 170 minutes, while the cortisol values at 105 minutes were greater ($P < 0.05$) than –10, 35, 70 and 170 minutes. During MGI/ HGL (isocaloric), serum cortisol concentrations after 35 minutes of exercise were lower ($P < 0.05$) than 70 and 105 minutes, while the cortisol values at 70 minutes of exercise were greater ($P < 0.05$) than 170 minutes (Tables 4.2 and 4.3).

DISCUSSION

Little research has characterized glucoregulatory hormonal responses following the consumption of CHO the hour prior to exercise based on the GI and GL. Given conflicting evidence regarding the benefits of consuming CHO during this pre-exercise time, research is warranted to clarify the efficacy of this nutritional strategy [9,12,15,16,22]. It was hypothesized in the present study that: (i) ingestion of LGI/ LGL compared to HGI/ HGL nutrition 60 minutes prior to exercise would elicit an augmented counter-regulatory response during prolonged cycling; (ii) ingestion of LGI/ LGL nutrition would negate the transient hypoglycemic and hyperinsulinemic perturbations observed following HGI nutrition at exercise onset. The major findings of this study are: (i) All meals resulted in transient hypoglycaemia and hyperinsulinemia immediately prior to exercise; (ii) ingestion of LGI/ LGL nutrition resulted in a greater glucagon response during exercise.

Regardless of GI/ GL, all meals resulted in a serum glucose nadir immediately prior to exercise. Concomitantly, hyperinsulinemia was also observed. Unexpectedly, the greatest nadir in serum glucose was observed in the LGI/ LGL trial. This finding is inconsistent with most previous studies when CHO was fed the hour before exercise either in solution (HGI-glucose versus LGI-fructose) [14,20,24,56-58] or in solid form (HGI-mashed potatoes versus LGI-lentils) [9,12,17,18] which have typically reported that LGI foods avert the hypoglyemia and hyerinsulinemia observed at exercise onset following HGI feedings. It was postulated that the insulinotropic effects of the additional protein and fat content in the LGI/ LGL meal likely mediated the greater transient hypoglycaemia observed in this trial. In addition to CHO, protein and fat stimulate secretion of the incretins, gastric inhibitory polypeptide (GIP) and glucagon like peptide 1 (GLP-1). Incretins potentiate glucose-dependent insulin secretion from β cells to facilitate the rapid

disposal of ingested nutrients [59,60]. Furthermore amino acids (AA) and fatty acids (FA) also exert direct and indirect (via the amplification of glucose-stimulated insulin secretion) insulinotropic effects on β cells following their absorption from the GI tract [61-63]. A concomitant increase in peripheral glucose disposal by insulin-sensitive tissue despite a lower flux of exogenous nutrients from the gut, could partially explain the glucose nadir following the LGI/ LGL meal [64]. In support of this contention, DeMarco and coworkers observed similar hypoglycemic and hyperinsulinemic responses following an LGI meal consumed 30 minutes pre-exercise [25]. Analogous, to the present study, the protein content of the LGI compared to the HGI meal was also greater in the former (18 versus 33 grams, respectively), and may have acted as a potent insulin secretagogue [25]. It remains less clear, why some solid LGI foods [25] but not others [9,12,17,18] elicit transient hypoglycemia pre-exercise. Differences in the insulinotropic potential of the test meals provided appears the most likely explanation, although contrasting levels of insulin sensitivity based on the training status of the subjects used could explain also the differential glycemia observed between studies [10,65,66].

Aside from the initial transient perturbations in glycemia, serum glucose concentrations declined with exercise duration in all trials, regardless of the pre-exercise meal GI/GL, and consistent with the observations of previous studies [9,13,18,24]. The fall in serum glucose is likely due to a negative imbalance between the rate of hepatic gluconeogenic output and peripheral glucose, which increases with increased exercise duration [67-70] While insulinemia was suppressed to basal levels by exercise in all trials, which serves to sensitize the liver to counter-regulatory mechanisms [71], glycemic and insulinemic responses initially tended to be higher during exercise in the LGI/ LGL trial.

This may be indicative of the continued absorption of nutrients from the GI tract and would corroborate the postulated slower rate of digestion and absorption of LGI foodstuff [72].

Serum NEFA concentrations were suppressed similarly following all test meals and progressively rose throughout exercise, but to a greater extent in the LGI/ LGL trial. It is unclear whether the augmented FA availability following the LGI/ LGL test meal was endogenous or exogenous in origin, given the absence of any lipolytic marker. Fat oxidation also increased in the LGI/ LGL and MGI/ MGL trials, evidenced by a reduction in RER with exercise duration. Such findings are consistent with those of previous studies [5,9,17,18,73]. As postulated by Randle's glucose-fatty acid cycle [74-76] elevated FA levels are known to reduce peripheral and hepatic insulin sensitivity, evidenced by impaired glucose uptake, glycolysis, glucose oxidation and glycogenesis [77-81]. FA's also facilitate glucose counter-regulation by augmenting hepatic gluconeogenic output [74,78-82]. Competitive inhibition of glucose uptake and oxidation due to the elevated NEFA concentrations observed in the LGI/ LGL trial may also explain the increased blood glucose response and reduced CHO oxidation observed. Furthermore, the insulinotropic effects of elevated NEFA concentrations could have also mediated the more prolonged insulinemia following the LGI/ LGL test meal [63].

In addition to augmented NEFA availability, serum glucagon concentrations were also elevated during exercise following the LGI/ LGL test meal, indicating a greater role of counter-regulatory mechanisms in maintaining euglycemia during this trial. Previous studies that have examined serum glucagon responses to CHO consumption the hour prior to exercise have observed suppressed glucagonemia compared to exercising in the

fasted state [83,84], but failed to find differences based on the GI [15,85]. Glucagon is transiently secreted from α cells in response to augmented autonomic neural signalling and circulating AA, along with attenuated concentrations of inhibitory paracrines (insulin and somatostatin) and hypoglycaemia [86-90]. Given the comparable markers of intensity (HR, VO_2) and insulinemia during exercise, the hyperglucagonemia observed during the LGI/ LGL trial is most likely attributable to a greater exercise-induced challenge to glucose homeostasis and/ or the glucagonotropic effects of the additional protein content in this meal [90-95]. Glucagon's physiological roles are antagonistic to those of insulin in maintaining euglycemia, stimulating hepatic glucogenic processes and the uptake of gluconeogenic precursors, whilst suppressing hepatic glycolytic and glycogenic pathways [86,91-93,95]. The heightened glucagon response observed following the LGI/ LGL meal implies a reliance on HGP to maintain euglycemia, likely due to a lower flux and availability of exogenous nutrients across the gut contributing to splanchnic glucose ouput. Low concentrations (18 pmol/l) of glucagon are known to stimulate HGP via glycogenolysis, while high concentrations (35 pmol/l) augment gluconeogenesis [71,82] suggesting that gluconeogenesis was the dominant process of HGP late in exercise during the LGI/ LGL trial.

Unexpectedly, serum cortisol responses were lower during the LGI/ LGL compared to the MGI/ MGL trial (only) which fails to substantiate the hyperglucagonemia observed in the former. This finding is inconsistent with previous studies that have reported no differences in cortisolemia during exercise, based on the GI [5,20,24,73]. The physiologic effects of glucagon and cortisol synergistically facilitate the Cori and glucose-alanine cycles, the latter through the provision of gluconeogenic

precursors (AA) from the periphery to the liver, while the former up-regulates hepatic glucogenic pathways [69,96-100]. Cortisol is secreted via the actions of the pituitary hormone adrenocorticotropic hormone (ACTH) on the adrenal cortex as part of the hypothalamic-pituitary-adrenal axis (HPA) [100,101]. Corticotropin releasing factor (CRF) is the primary stimulus for ACTH secretion, although additional secretagogues act during exercise including (most potently) arginine vasopressin (AVP), catecholamines, angiotensin and (possibly) hypovolemia, interleukins or blood lactate [100,102-104]. Different blood lactate concentrations might have mediated the contrasting cortisolemia observed in the LGI/ LGL and MGI/ MGL trials. Hyperglucagonemia in the former may also have facilitated greater hepatic uptake of blood lactate, diminishing its stimulatory effect on ACTH secretion. Alternatively, the lower energy provided in the MGI/ MGL trial might have resulted in a lower provision of exogenous gluconeogenic precursors, necessitating hypercortisolemia to maintain adequate provision of substrates for the glucose-alanine cycle. Lastly, given the small sample size used in the present study, the possibility of a type 1 error must not be precluded. Regardless of the mediators of the hypercortisolemia observed in the MGI/ MGL trial, it most likely had little physiologic significance during 105 minutes of exercise however, as the actions of cortisol take several to become evident [91,105-107].

Understanding of the differential benefits of consuming CHO foods based on the GI has been greatly enhanced through the use of stable isotopes. The literature suggests a lower bioavailability of LGI compared to HGI foods consumed the hour before based on lower rates of appearance during exercise [9]. Furthermore lower levels of oxidation of exogenous CHO and augmented endogenous CHO utilization has also been consistently

reported following the consumption of LGI compared to HGI sugar solutions in the hours prior to [27,28] or during exercise [108-111]. This supports the contention that the hyperglucagonemia observed in the LGI/ LGL trial was indicative of a higher rate of HGP and depletion of endogenous CHO during exercise. Many factors affect a foods rate of digestion and absorptive and its bioavailability during exercise, including the type of CHO, the presence of dietary fiber, fat or protein and food form amongst others [35,112-117]. Metabolic availability however is also likely an issue with LGI food sources given their high fructose or galactose content. Both sugars require assimilation, in the liver via the Leloir pathway and fructose metabolism, respectively, before entry into gluconeogenisis. These processes would further delay their availability as fuels during exercise [118-121].

A major novelty of this study was the use of nutrition bars providing a balanced mixture of macronutrients with validated GI and GL values. It was suggested that this would negate some criticisms [35,36,39,41,44,46,121-123] and limitations of previous studies [9,12,13,17,18,25,28,47] that have investigated the GI's predictive capability for mixed meals, by calculating a meal GI (MGI) based on the GI values of the constituent single foods [11]. Furthermore nutrition bars are a convenient and readily accessible CHO food-source consumed by athletes before, during and after exercise. In conclusion, the findings of this study show that pre-exercise ingestion of LGI/ LGL nutrition results in augmented serum NEFA availability, hyperglucagonemia and increased fat oxidation during exercise compared to MGI/ HGL nutrition. Despite these differences, serum glucose was maintained similarly between the three trials. LGI feedings containing significant quantities of fat and protein also fail to negate the transient hypglycemia and hyperinsulinemia observed at exercise onset in endurance trained individuals.

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Table 4.1. Nutritional intake for the three test meals.

	LGI/ LGL (1g CHO/ kg)	MGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
Volume (g)	168.45 ± 8.35	97.79 ± 4.81	155.73 ± 7.66
Total Carbohydrate (g)	77.49 ± 3.84	77.53 ± 3.82	123.46 ± 6.07
Dietary Fibre (g)	10.11 ± 0.50	7.05 ± 0.35	11.22 ± 0.55
Sugars (g)	50.54 ± 2.50	31.72 ± 1.56	50.51 ± 2.48
Total Useful Carbohydrate (g)	67.38 ± 3.34	70.48 ± 3.47	112.24 ± 5.52
Protein (g)	43.8 ± 2.17	12.33 ± 0.61	19.64 ± 0.97
Total Fat (g)	26.95 ± 1.34	7.93 ± 0.39	12.63 ± 0.62
Saturated Fat (g)	10.11 ± 0.50	1.76 ± 0.09	2.81 ± 0.14
Calories (kcal)	673.8 ± 33.39	422.88 ± 20.81	673.44 ± 33.11
GI (%)	27*	60**	60**
GL (g)	18.19 ± 0.90	42.29 ± 2.08	67.34 ± 3.31

Values are means ± S.D. * = Clinically validated GI; ** = Estimated GI

Table 4.2: Changes in total serum responses over time following the three test meals.

	Time (min)	-80	-10	35	70	105	170
Glucose (mmol/l)	LGI/ LGL (1g CHO/ kg)	4.68 ± 0.47	3.12 ± 0.77^{acdef}	4.81 ± 1.03	4.93 ± 0.87^{f}	3.74 ± 0.94^{ad}	4.19 ± 0.83
	MGI/ MGL (1g CHO/ kg)	4.72 ± 0.66	3.56 ± 1.03^{ac}	4.88 ± 1.02^{d}	4.22 ± 0.83	3.94 ± 0.59^{ac}	4.49 ± 0.49
	MGI/ HGL (isocaloric)	4.57 ± 0.27	3.63 ± 1.29^{c}	4.92 ± 0.79	4.35 ± 0.81	4.03 ± 0.55^{ac}	4.50 ± 0.91
NEFA (mmol/l)	LGI/ LGL (1g CHO/ kg)	0.51 ± 0.14	0.30 ± 0.07	0.40 ± 0.14^{e}	0.67 ± 0.25^{bc}	0.91 ± 0.39^{abc}	1.32 ± 0.32^{abcd}
	MGI/ MGL (1g CHO/ kg)	0.61 ± 0.34	0.29 ± 0.17^{a}	0.27 ± 0.13^{a}	0.50 ± 0.17^{bc}	0.84 ± 0.28^{bcd}	1.43 ± 0.37^{abcde}
	MGI/ HGL (isocaloric)	0.45 ± 0.12	0.24 ± 0.05^{a}	0.24 ± 0.03^{a}	0.40 ± 0.10^{bc}	0.74 ± 0.22^{abcd}	1.07 ± 0.29^{abcd}
Insulin (uU/ml)	LGI/ LGL (1g CHO/ kg)	3.87 ± 1.57^{e}	16.24 ± 6.08^{acdef}	6.75 ± 2.79^{def}	3.73 ± 2.11^{e}	2.35 ± 1.16	3.16 ± 1.38^{e}
	MGI/ MGL (1g CHO/ kg)	4.57 ± 1.31^{cdef}	22.65 ± 22.13^{acdef}	2.90 ± 1.20^{e}	2.08 ± 0.91^{e}	1.41 ± 0.83	2.70 ± 1.30
	MGI/ HGL (isocaloric)	5.32 ± 1.69^{de}	20.48 ± 9.80^{acdef}	5.14 ± 1.16^{de}	3.40 ± 0.42^{e}	2.11 ± 0.37	4.55 ± 3.46
Glucagon (pmol/l)	LGI/ LGL (1g CHO/ kg)	16.42 ± 4.10	22.88 ± 10.91	27.82 ± 7.21^{af}	30.81 ± 7.90^{abcf}	31.78 ± 8.09^{acf}	20.46 ± 7.15
	MGI/ MGL (1g CHO/ kg)	13.98 ± 5.51	17.79 ± 5.75^{a}	18.47 ± 6.81^{af}	26.45 ± 14.11^{abc}	24.02 ± 8.48^{abcf}	19.11 ± 6.78^{a}
	MGI/ HGL (isocaloric)	16.84 ± 3.09	21.17 ± 4.54^{a}	24.71 ± 4.57^{abf}	20.89 ± 9.12	24.60 ± 6.00^{a}	18.87 ± 3.12
Insulin/ glucagon (molar ratio)	LGI/ LGL (1g CHO/ kg)	1.88 ± 1.18	6.56 ± 3.99^{acdef}	1.89 ± 1.05	0.99 ± 0.76^{ac}	0.62 ± 0.49^{acdf}	1.19 ± 0.54
	MGI/ MGL (1g CHO/ kg)	2.56 ± 1.05	8.45 ± 6.13^{acdef}	1.26 ± 0.75^{a}	0.73 ± 0.66^{ac}	0.49 ± 0.37^{acf}	1.01 ± 0.32^{a}
	MGI/ HGL (isocaloric)	2.36 ± 0.53	7.16 ± 2.77^{acdef}	1.52 ± 0.28^{a}	1.36 ± 0.64^{a}	0.64 ± 0.09^{acd}	1.89 ± 1.76
Cortisol (ug/dl)	LGI/ LGL (1g CHO/ kg)	18.30 ± 3.61^{i}	17.42 ± 6.15	13.50 ± 3.58	12.74 ± 4.20	16.16 ± 6.03	13.43 ± 4.62
	MGI/ MGL (1g CHO/ kg)	17.94 ± 2.31^{c}	14.86 ± 2.56	12.96 ± 2.69	15.80 ± 3.18^{c}	20.92 ± 6.96^{bcdf}	15.87 ± 3.52^{c}
	MGI/ HGL (isocaloric)	16.00 ± 3.45	14.38 ± 2.89	13.90 ± 3.62	18.67 ± 6.73^{ci}	19.60 ± 6.94^{c}	16.11 ± 4.25

Values are means ± S.D. -80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. a = significantly different from -80 min; b = significantly different from -10 min; c = significantly different from 35 min; d = significantly different from 70 min; e = significantly different from 105 min; f = significantly different from 170 min. $P < 0.05$.

Table 4.3: Changes in incremental serum responses over time following the three test meals.

	Time (min)	-10	35	70	105	170
Glucose (mmol/l)	LGI/ LGL (1g CHO/ kg)	-1.58 ± 0.58^{cdef}	0.13 ± 0.64	0.25 ± 0.56^{ef}	-0.94 ± 0.85	-0.49 ± 0.50
	MGI/ MGL (1g CHO/ kg)	-1.16 ± 1.02	0.16 ± 1.09^{bde}	-0.5 ± 1.07	-0.78 ± 0.90	-0.23 ± 0.57
	MGI/ HGL (isocaloric)	-0.95 ± 1.35	0.35 ± 0.75^{be}	-0.23 ± 0.81	-0.55 ± 0.49	-0.07 ± 0.74
NEFA (mmol/l)	LGI/ LGL (1g CHO/ kg)	-0.21 ± 0.20^{cdef}	-0.11 ± 0.23^{def}	0.15 ± 0.26^{f}	0.39 ± 0.42	0.81 ± 0.22
	MGI/ MGL (1g CHO/ kg)	-0.31 ± 0.31^{def}	-0.33 ± 0.41^{def}	-0.11 ± 0.43^{ef}	0.23 ± 0.50	0.83 ± 0.22
	MGI/ HGL (isocaloric)	-0.21 ± 0.12^{def}	-0.21 ± 0.12^{def}	-0.04 ± 0.11^{ef}	0.29 ± 0.19	0.62 ± 0.23
Insulin (uU/ml)	LGI/ LGL (1g CHO/ kg)	12.36 ± 5.09^{cdef}	2.88 ± 2.74^{def}	-0.14 ± 1.53^{e}	-1.53 ± 0.94	-0.71 ± 1.43^{e}
	MGI/ MGL (1g CHO/ kg)	18.08 ± 21.50^{cdef}	-1.67 ± 0.75^{e}	-2.49 ± 1.40	-3.16 ± 0.90	-1.87 ± 0.61^{e}
	MGI/ HGL (isocaloric)	15.16 ± 8.49^{cdef}	-0.18 ± 2.07^{de}	-1.92 ± 1.92^{e}	-3.21 ± 1.74	-0.78 ± 2.42
Glucagon (pmol/l)	LGI/ LGL (1g CHO/ kg)	6.46 ± 9.38	11.40 ± 5.34^{f}	14.39 ± 4.76^{bcf}	15.35 ± 6.34^{cf}	4.04 ± 4.86
	MGI/ MGL (1g CHO/ kg)	3.81 ± 2.61	4.49 ± 2.79^{f}	12.48 ± 9.76^{bc}	10.04 ± 4.15^{bcf}	5.14 ± 3.07
	MGI/ HGL (isocaloric)	4.33 ± 3.43	7.87 ± 3.94^{bf}	4.05 ± 9.87	7.75 ± 4.13	5.02 ± 5.06
Insulin/ glucagon (molar ratio)	LGI/ LGL (1g CHO/ kg)	4.68 ± 3.20^{cdef}	0.01 ± 0.92	-0.90 ± 0.63^{c}	-1.26 ± 0.77^{cdf}	-0.69 ± 0.86
	MGI/ MGL (1g CHO/ kg)	5.89 ± 6.41^{cdef}	-1.30 ± 0.56	-1.82 ± 0.98^{c}	-2.06 ± 0.77^{cf}	-1.55 ± 0.98
	MGI/ HGL (isocaloric)	4.80 ± 2.48^{cdef}	-0.83 ± 0.44	-1.00 ± 0.59	-1.72 ± 0.58^{cd}	-0.47 ± 1.66
Cortisol (ug/dl)	LGI/ LGL (1g CHO/ kg)	-0.89 ± 3.88	-4.80 ± 4.11	-5.57 ± 6.25	-2.15 ± 8.12	-4.87 ± 6.46
	MGI/ MGL (1g CHO/ kg)	-3.08 ± 3.19	-4.98 ± 3.18^{df}	-2.13 ± 3.69	2.98 ± 7.29^{bcdf}	-2.06 ± 4.23
	MGI/ HGL (isocaloric)	-1.62 ± 3.84	-2.11 ± 6.65	2.66 ± 9.49^{cf}	3.60 ± 9.12^{c}	0.11 ± 7.44

Values are means ± S.D. -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. b = significantly different from -10 min; c = significantly different from 35 min; d = significantly different from 70 min; e = significantly different from 105 min; f = significantly different from 170 min. $P < 0.05$.

Table 4.4. Total areas under the curve (tAUC) for glucose, NEFA, insulin, glucagons, insulin/ glucagon ratio and cortisol responses to the three test meals.

	LGI/ LGL (1g CHO/ kg)	MGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
Glucose ($mmol \cdot l^{-1} min^{-1}$)	1030.96 ± 173.98	1054.94 ± 151.06	1065.00 ± 159.97
NEFA ($mmol \cdot l^{-1} min^{-1}$)	163.00 ± 42.58	154.77 ± 36.52	124.70 ± 22.50
Insulin ($uU \cdot ml^{-1} min^{-1}$)	1690.05 ± 651.40	1809.35 ± 1345.71	1941.99 ± 722.44
Glucagon ($pmol \cdot l^{-1} min^{-1}$)	6335.20 ± 1619.96	4998.85 ± 1781.04	5369.78 ± 981.82
Insulin/ glucagon ($mol \cdot l^{-1} min^{-1}$)	622.84 ± 329.50	708.71 ± 354.24	696.26 ± 227.65
Cortisol ($ug \cdot dl^{-1} min^{-1}$)	3872.45 ± 662.63	4115.20 ± 747.65	4100.33 ± 728.14

Values are means ± S.D.

Table 4.5. Incremental areas under the curve (iAUC) for glucose, NEFA, insulin, glucagons, insulin/ glucagon ratio and cortisol responses to the three test meals.

	LGI/ LGL (1g CHO/ kg)	MGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
Glucose ($mmol \cdot l^{-1} min^{-1}$)	15.82 ± 13.10	22.33 ± 35.14	43.06 ± 46.96
NEFA ($mmol \cdot l^{-1} min^{-1}$)	53.40 ± 38.47	45.29 ± 31.71	34.47 ± 9.21
Insulin ($uU \cdot ml^{-1} min^{-1}$)	848.68 ± 414.63	1004.74 ± 1222.62	878.37 ± 451.68
Glucagon ($pmol \cdot l^{-1} min^{-1}$)	2263.27 ± 916.36	1504.52 ± 504.62	1218.15 ± 766.32
Insulin/ glucagon ($mol \cdot l^{-1} min^{-1}$)	284.68 ± 156.08	368.56 ± 320.80	148.36 ± 265.13
Cortisol ($ug \cdot dl^{-1} min^{-1}$)	150.32 ± 222.39	218.42 ± 381.60	634.92 ± 929.20

Values are means ± S.D.

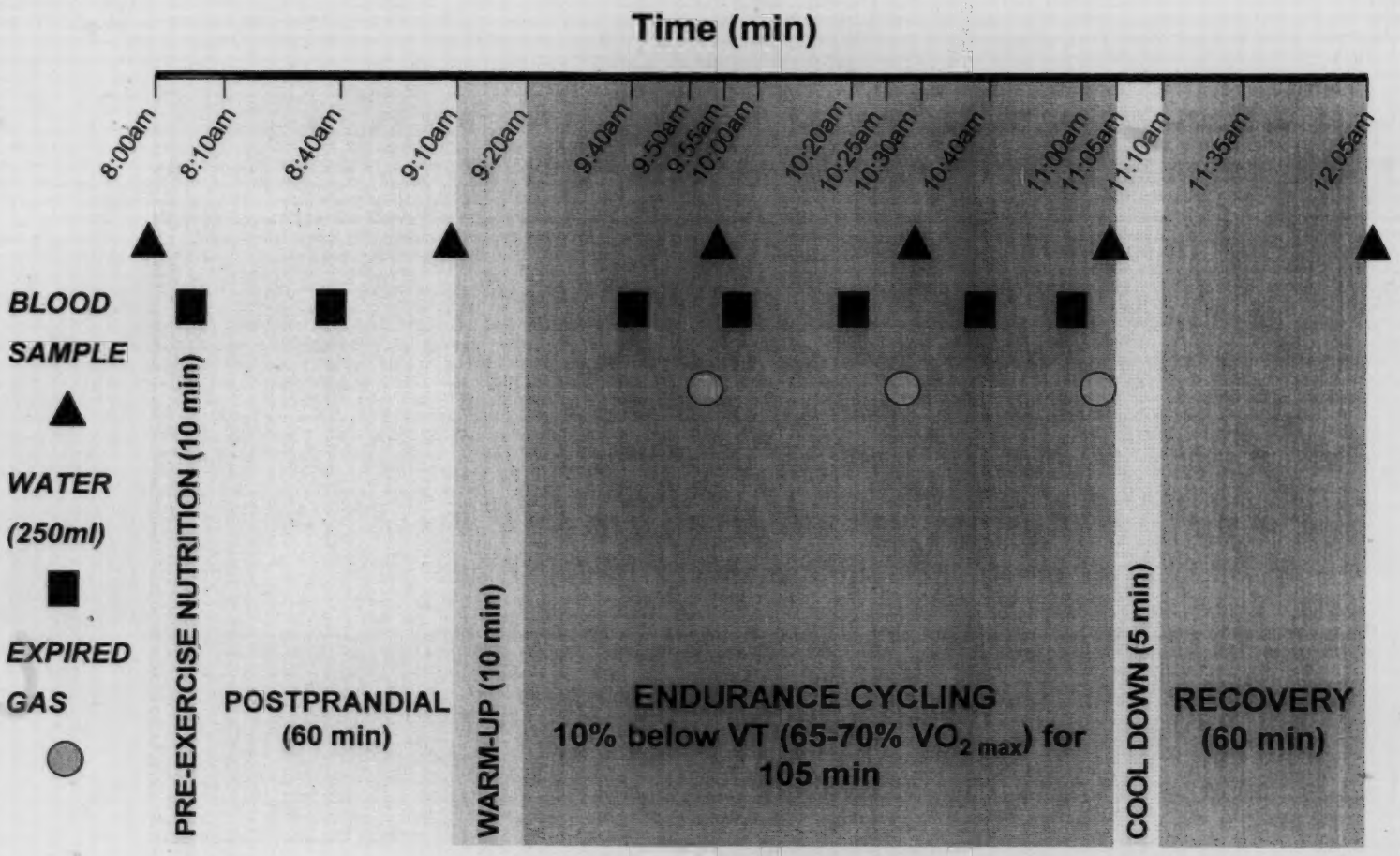

Figure 4.1: Trial procedure

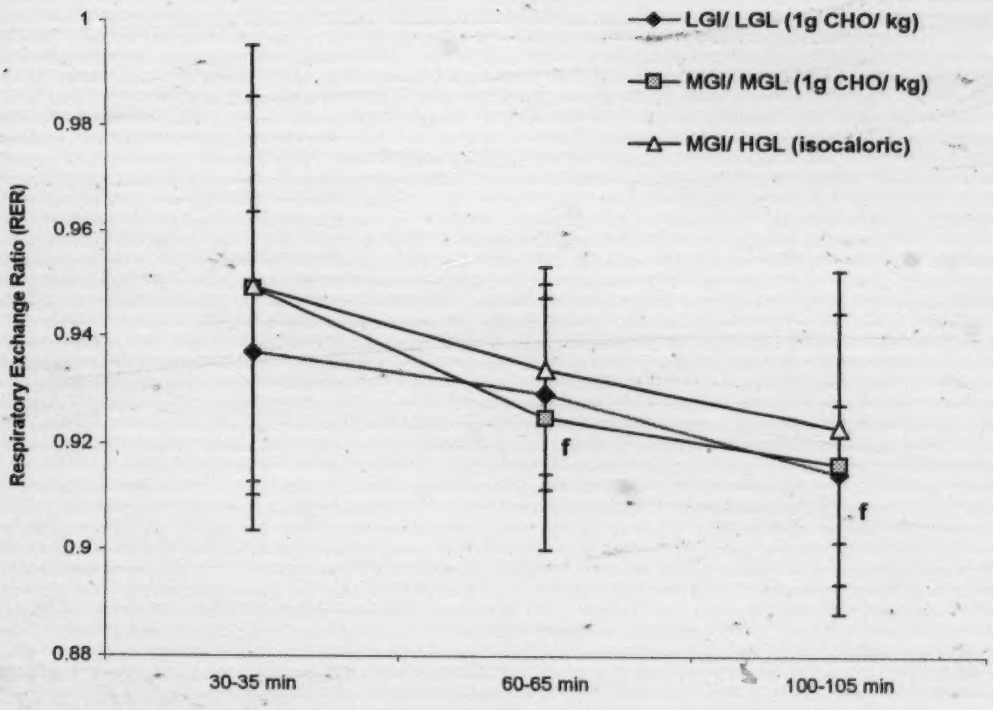

Figure 4.2: Respiratory Exchange Ratio (RER) during 105 minutes of cycling following the three test meals. Values are means ± S.D. c = significantly different from 30-35 min. $P < 0.05$.

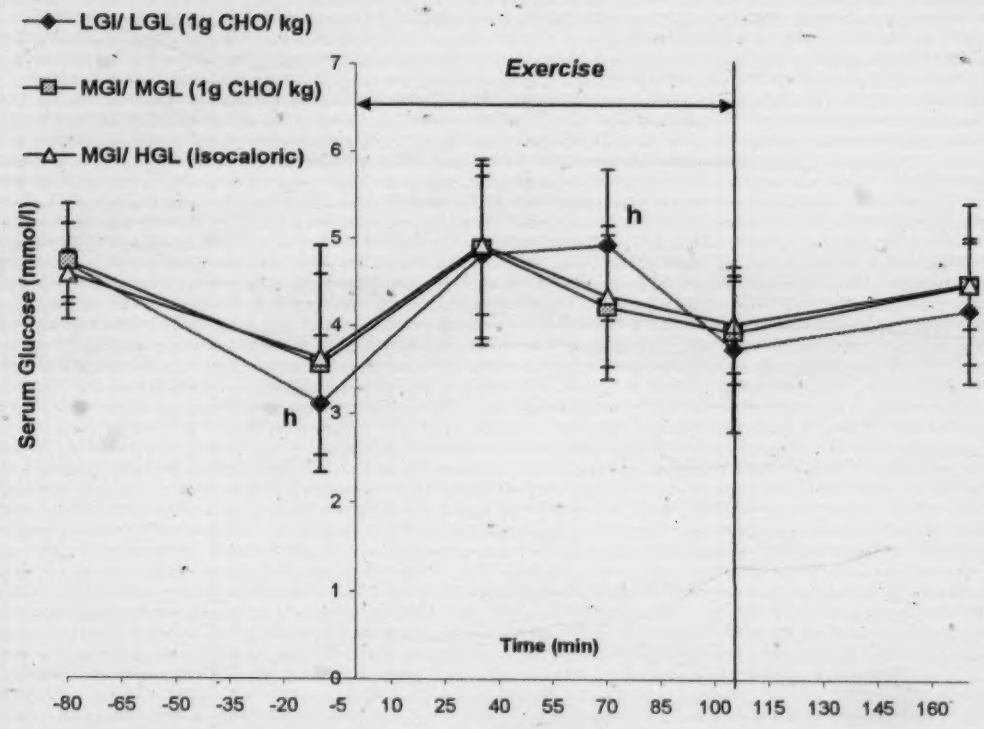

Figure 4.3: Changes in the total serum glucose response over time between the three test meals.-80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. Values are means ± S.D. h = significantly different from MGI/ MGL (1g CHO/ kg). $P < 0.05$.

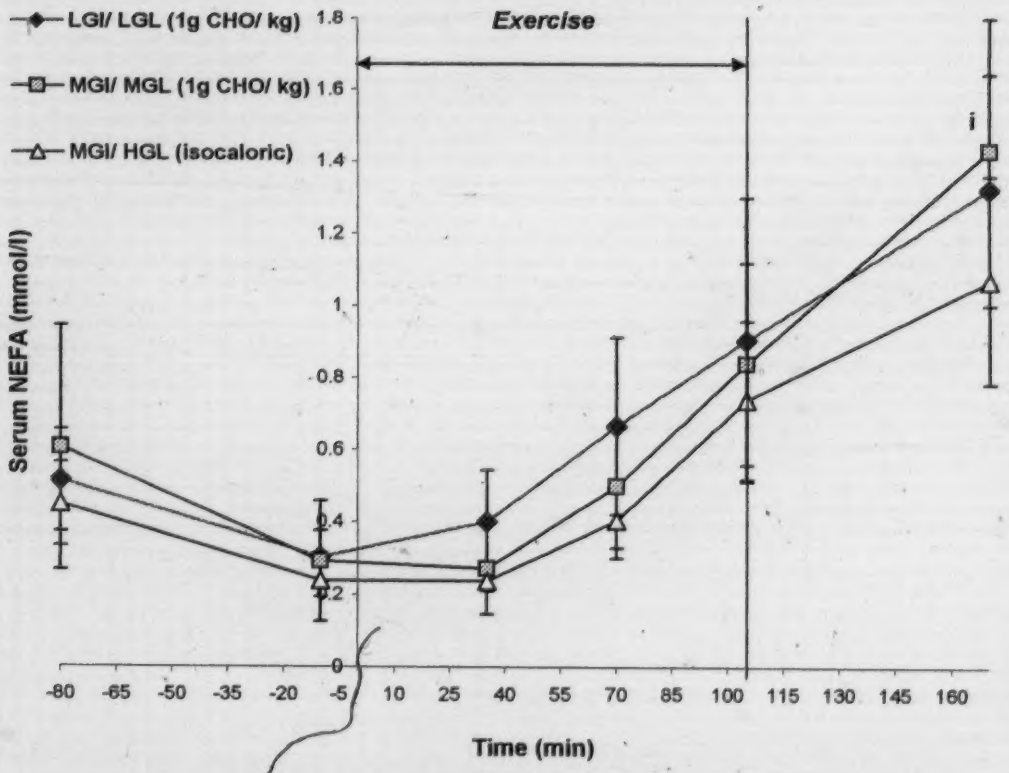

Figure 4.4: Changes in the total NEFA response over time between the three test meals.-80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. Values are means ± S.D. i = significantly different from MGI/ HGL (isocaloric). $P < 0.05$.

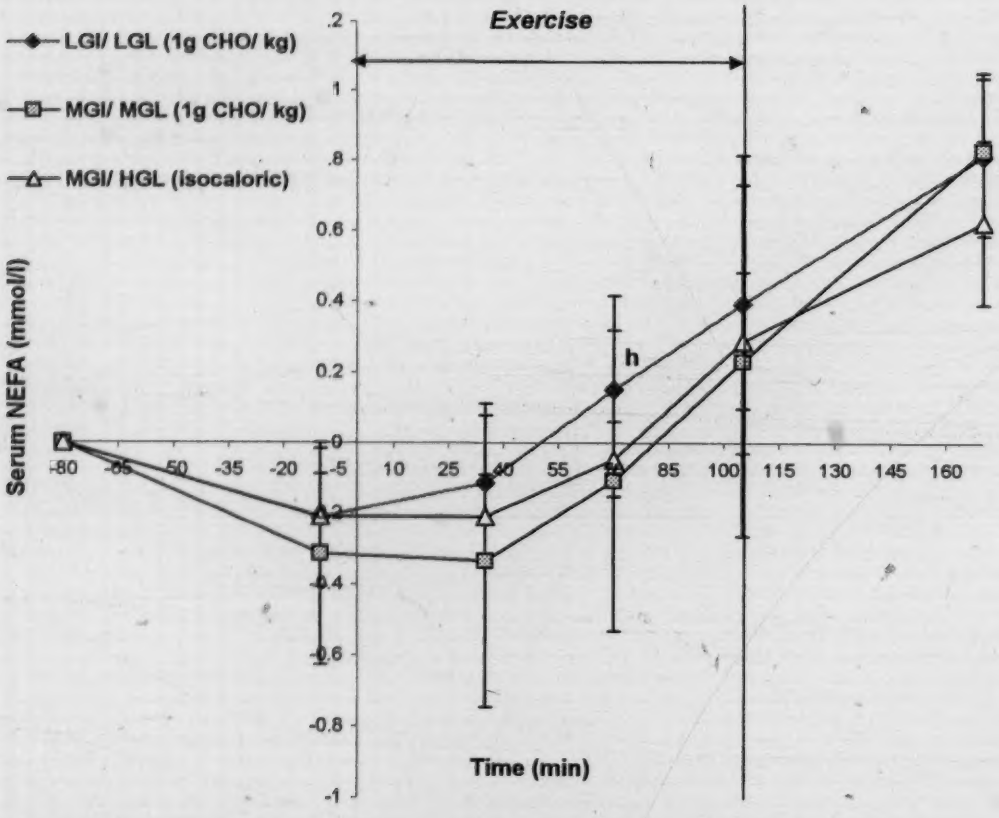

Figure 4.5: Changes in the incremental NEFA response over time between the three test meals.-80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. Values are means ± S.D. h = significantly different from MGI/ MGL (1g CHO/ kg). $P < 0.05$.

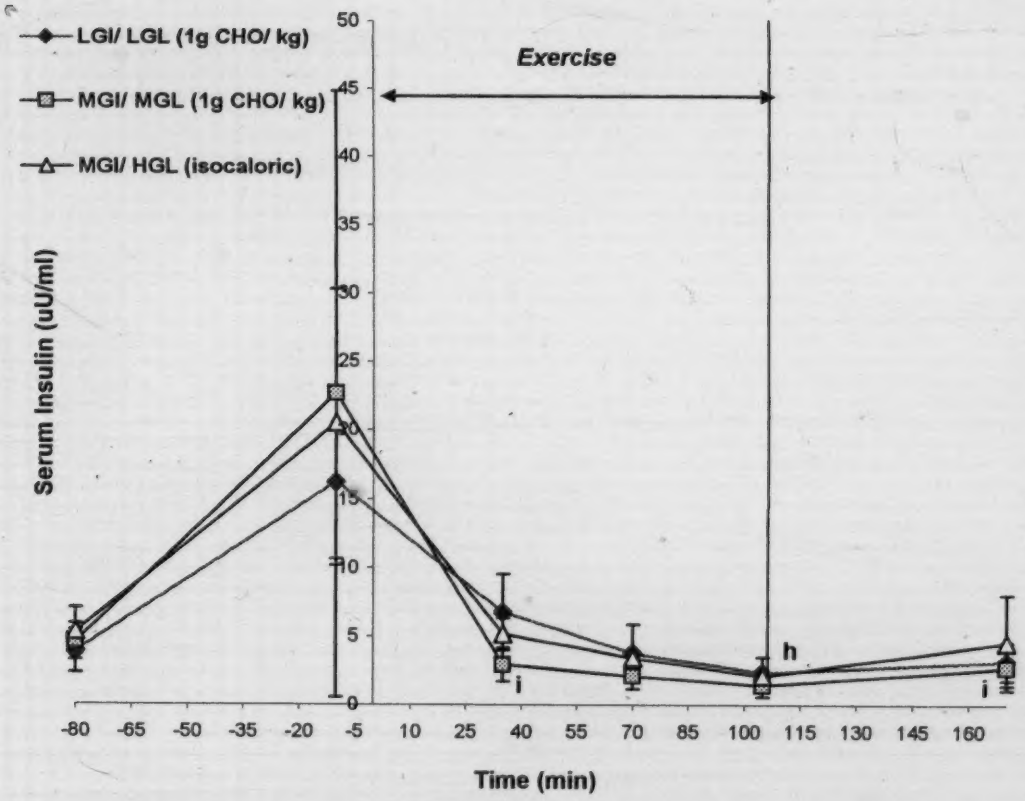

Figure 4.6: Changes in the total insulin response over time between the three test meals.-80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. Values are means ± S.D. h = significantly different from MGI/ MGL (1g CHO/ kg); i = significantly different from MGI/ HGL (isocaloric). $P < 0.05$.

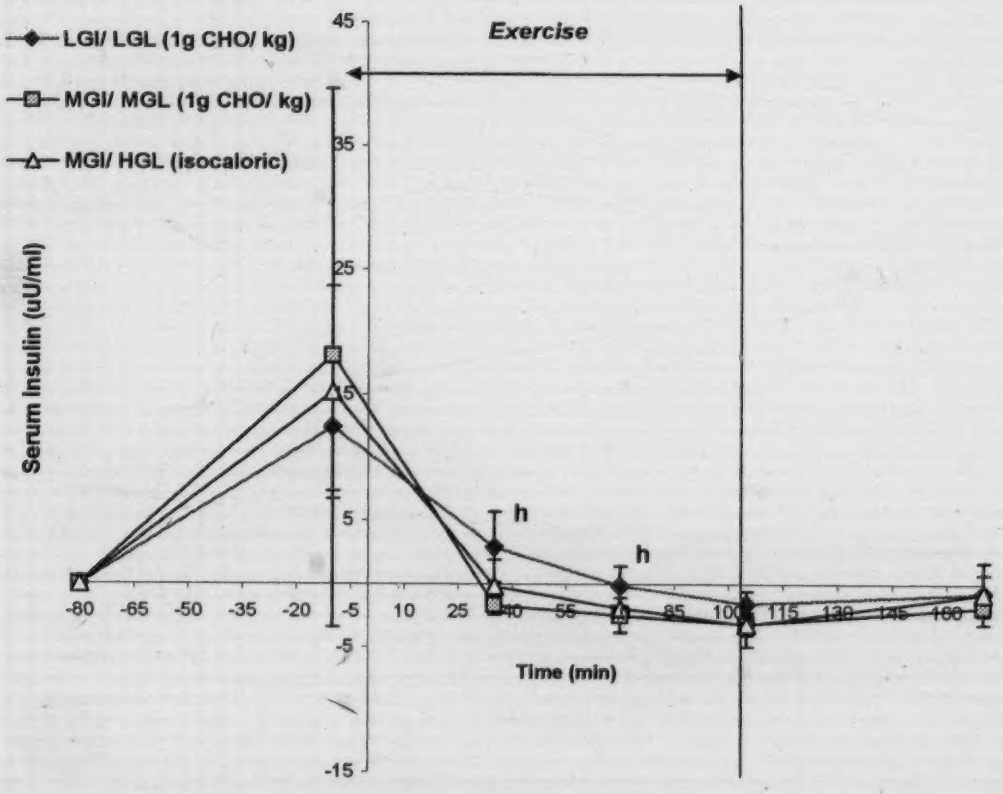

Figure 4.7: Changes in the incremental insulin response over time between the three test meals.-80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. Values are means ± S.D. h = significantly different from MGI/ MGL (1g CHO/ kg). $P < 0.05$.

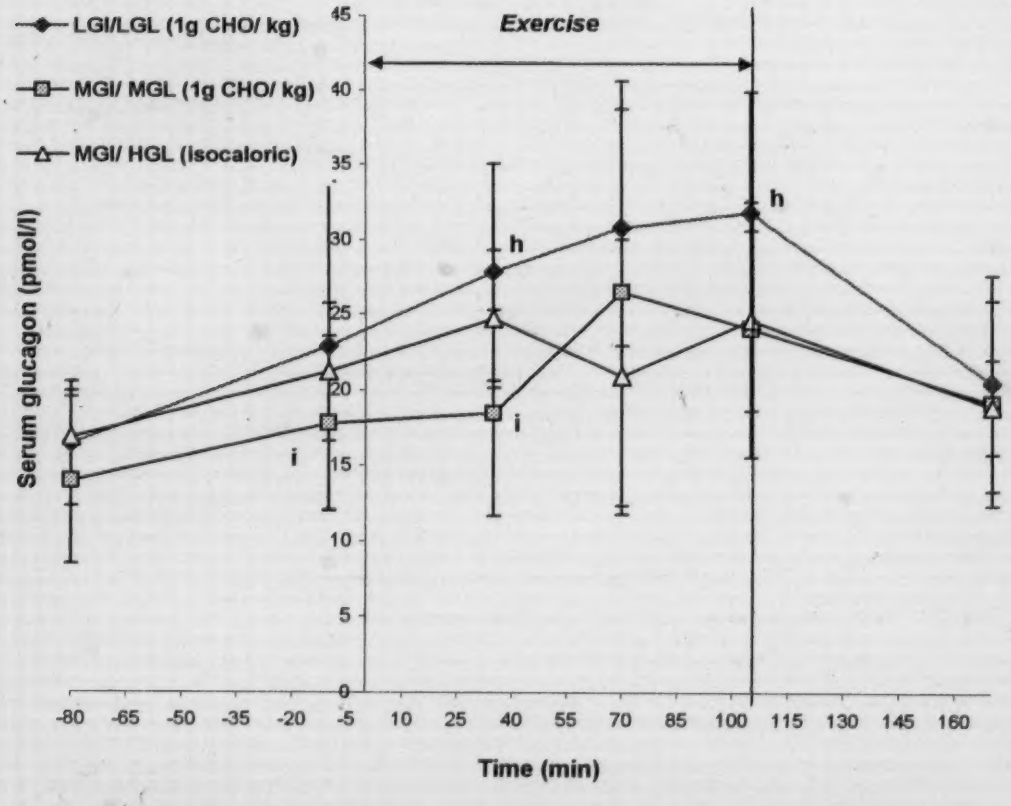

Figure 4.8: Changes in the total glucagon response over time between the three test meals.-80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. Values are means ± S.D. h = significantly different from MGI/ MGL (1g CHO/ kg); i= significantly different from trial MGI/ HGL (isocaloric). $P < 0.05$.

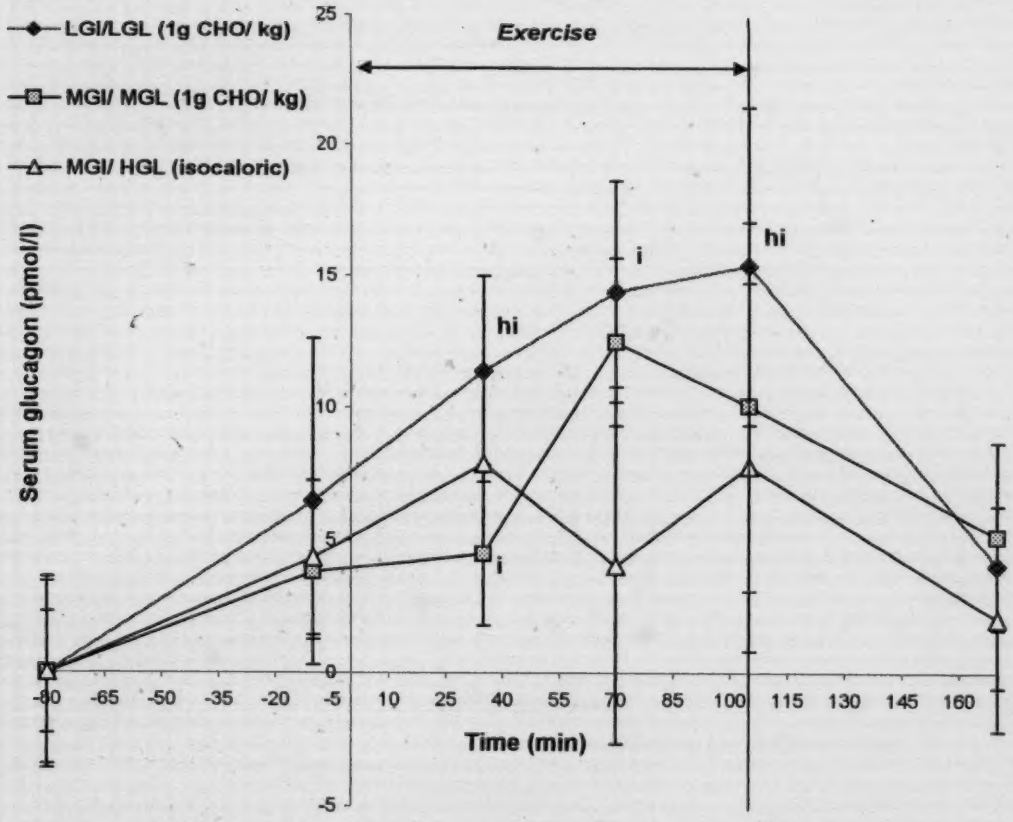

Figure 4.9: Changes in the incremental glucagon response over time between the three test meals.-80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. Values are means ± S.D. h = significantly different from MGI/ MGL (1g CHO/ kg); i = significantly different from MGI/ HGL (isocaloric). $P < 0.05$.

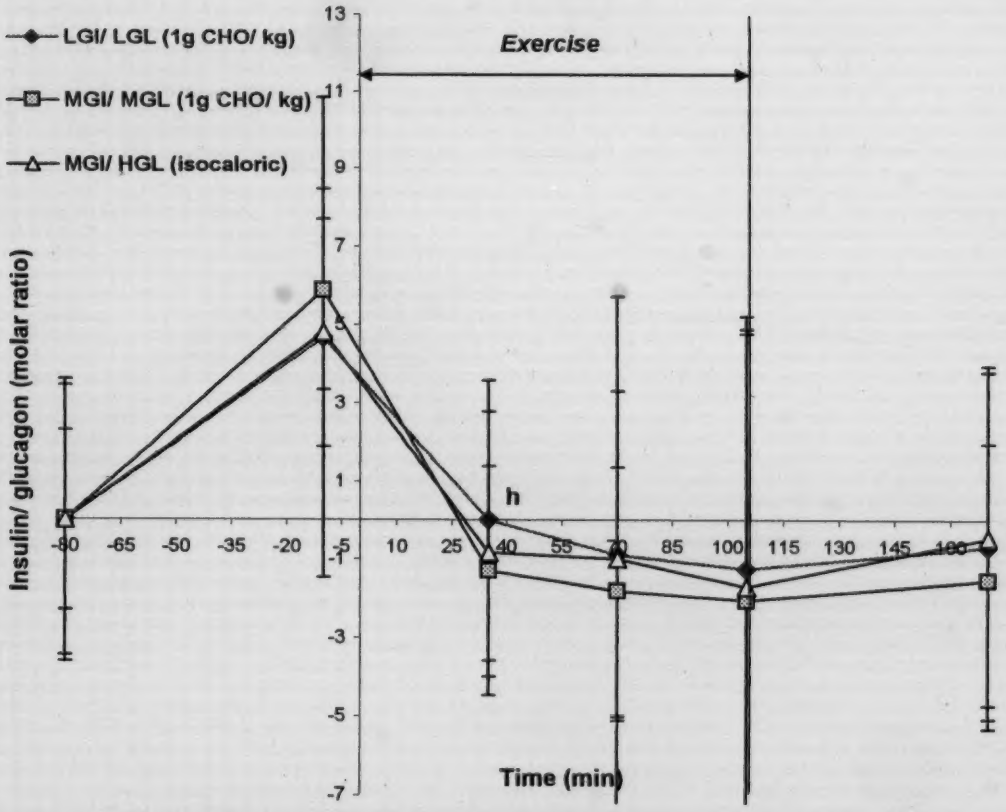

Figure 4.10: Changes in the incremental insulin/ glucagon response over time between the three test meals.-80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. Values are means ± S.D. h= significantly different from MGI/ MGL (1g CHO/ kg). $P < 0.05$.

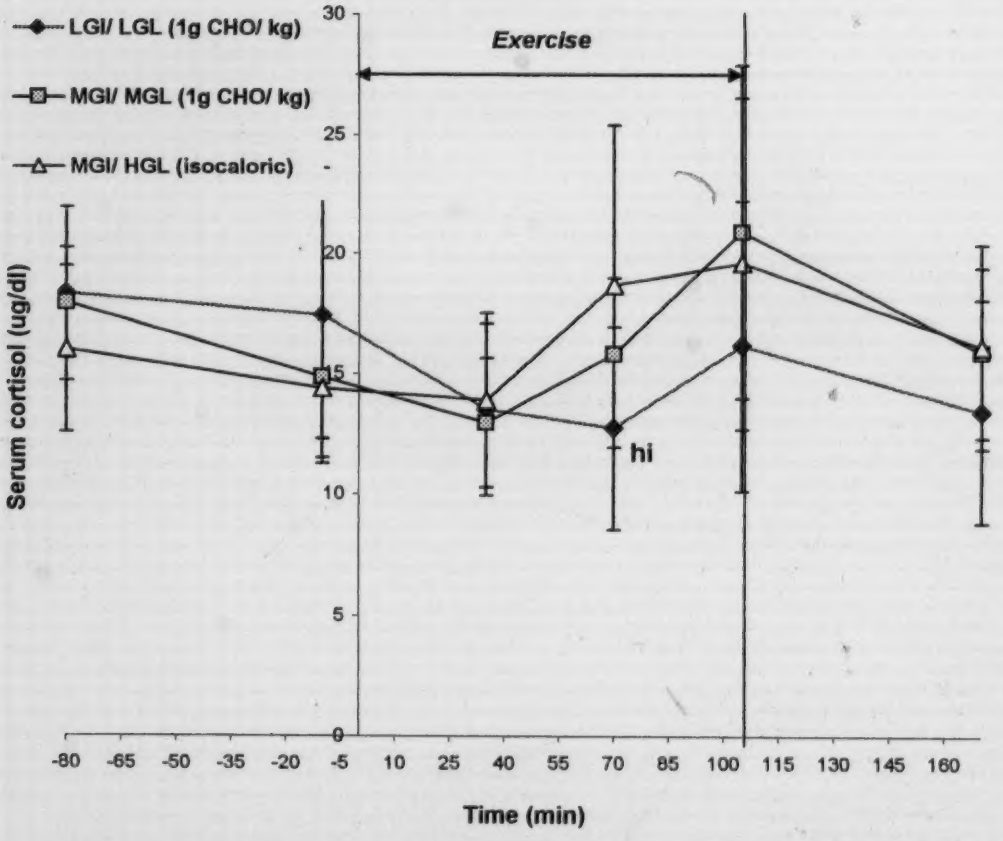

Figure 4.11: Changes in the total cortisol response over time between the three test meals.-80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. Values are means ± S.D. h = significantly different from MGI/ MGL (1g CHO/ kg). i = significantly different from MGI/ HGL (isocaloric). $P < 0.05$.

CHAPTER 5 – CONCLUSIONS AND FUTURE DIRECTIONS

This thesis focused on the effects of the glycemic index (GI) and glycemic load (GI) on metabolic and glucoregulatory hormonal responses at rest and during exercise. Relative to low GI (LGI) meals, the consumption of high GI (HGI) foodstuff elicits exaggerated hyperinsulinemia and hypoglucagonemia during the early postprandial period at rest in obese individuals [1-3]. In sports nutrition some authors have advocated the consumption of LGI carbohydrate (CHO) foods the hour before exercise [4], but evidence suggests that such food sources are limited in their bioavailability for utilization during exercise [5-7]. Few, if any studies have characterized metabolic and gluco-regulatory hormonal responses based on the GI and GL in healthy individuals at rest or during exercise.

The GI's predictive capability for mixed meals via the calculation of a meal GI (MGI) using values from each constituent single food [8] has been questioned given the myriad of factors that affect postprandial glycemia [9-15]. Some evidence supports the GI's predictive capabilities in this context [8,16,17], while others have not [18-22]. Studies examining the effect of consuming CHO the hour prior to exercise [7,23-28] and at rest [1-3,16,17,21] using the GI have frequently used mixed meals despite this debate. A major novelty of this study was the use of nutrition bars providing a balanced mixture of macronutrients with validated GI and GL values. Nutrition bars are a convenient and readily accessible food-source for individuals at rest and during exercise. It was suggested that given their known GI and GL values [29-31] the use of this food-source would facilitate more valid investigation into these indices efficacy in characterizing the metabolic responses to mixed macronutrient meals. It is also difficult to find studies that

have characterized the metabolic and gluco-regulatory hormonal responses to the GI and GL in nutrition bar form at rest or during exercise.

Study one examined the effects of the GI and GL on the glucose and gluco-regulatory hormonal responses at rest. Healthy males were asked to attend the laboratory on three occasions, to consume a LGI/ LGL, HGI/ MGL or MGI/ HGL meal. Serum glucose responses were similar in all trials. Insulin levels were lower while glucagons levels were greater following the LGI/ LGL meal. NEFA concentrations were suppressed in all trials, but to a greater extent following the MGI/ HGL test meal. The insulin/ glucagon molar ratio was also greatest during the MGI/ HGL trial indicating a greater challenge to glucose homeostatic mechanisms following this test meal. While the GI and GL appear equally capable of predicting the acute insulinemia in response to mixed meals, the GL better corresponded to the insulinogenic effects postprandially.

Obese patients fed HGI compared to LGI meals have been shown to exhibit greater glycemic and insulinemic responses but attenuated glucagon concentrations during the early to mid postprandial period (0-4 hours) [1,3]. To date, there appears to be only one study that has directly determined the gluco-regulatory hormonal responses to the GI in healthy non-obese individuals. Wee et al reported that HGI meals resulted in elevated glycemic and insulinemic responses but suppressed glucagonemia and FFA concentrations compared to LGI foodstuff [2]. Less evidence exists examining the effects of acute feedings using the GL on glucose regulation. Brand-Miller et al demonstrated that the GL of test meals was linearly related to incremental areas under both the glycemic and insulinemic response curves [32]. Evidence also suggests that while both the GI and GL are good predictors of glycemia and insulinogenic demand, the GL better

accounts for the variations observed following different test meals [33]. No studies however, have previously examined the glucoregulatory hormonal responses to the GI and GL in healthy non-obese individuals.

Recently, a conceptual model was provided to characterise the acute physiological effects of the GI. During the early postprandial period (~ 2 hours) Ludwig et al postulated that the rapid absorption of a HGI meal elevates glycemia (~30 minutes in healthy individuals) by up to twice the magnitude of the rise observed following LGI meals [1-3,35,36]. Such a pronounced transient increase in blood glucose along with augmented concentrations of gut incretins potently stimulate insulin and inhibit glucagon secretion from pancreatic β and α cells, respectively [37,38]. A dramatic increase in the insulin: glucagon ratio following a HGI meal exaggerates anabolic responses in insulin-responsive tissues, including nutrient uptake, glycogenesis, lipogenesis and proteogenesis whilst suppressing the catabolic actions of glycogenolysis, gluconeogenesis and lipolysis [35,39]. LGI meals however, due to their slower rate of digestion elicit lower glycemic and insulinemic responses, enable the continued secretion of glucagon and the maintenance of glycogenolytic, gluconeogenic and lipolytic processes, evidence by higher levels of circulating FFA and glycerol [1,2,35,37].

Study two examined the effects of the GI and GL on the glucose and gluco-regulatory hormonal responses during prolonged cycling. Highly trained male cyclists were asked to exercise for 105 minutes on three occasions, 60 minutes after the consumption of either a LGI/ LGL, MGI/ MGL or MGI/ HGL meal. All trials resulted in transient hypoglycemia and hyperinsulinemia immediately prior to exercise. Serum NEFA concentrations were suppressed similarly following all test meals and

progressively rose throughout exercise, but to a greater extent in the LGI/ LGL trial. Serum glucagon concentrations were also elevated during exercise following the LGI/ LGL test meal.

Little research has previously examined glucoregulatory hormonal responses based on the GI and GL to CHO foods ingested the hour prior to exercise. Prior investigations that determined serum glucagon responses following the consumption of CHO before prolonged activity have reported suppressed glucagonemia compared to exercising in the fasted state [40,41] but failed to find differences based on the GI when comparing HGI-glucose to LGI-fructose solutions [42,43]. Stable isotope studies have generally suggested a lower bioavailability of LGI foods based on attenuated rates of appearance relative to HGI meals during exercise [7]. A reduction in exogenous and augmentation of endogenous CHO oxidation has also been typically been reported following the consumption of LGI compared to HGI solutions in the hours prior to [5,6] or during exercise [44-47]. The present study is the first to report differential glucagonemia based on the GI during exercise following the consumption of CHO the hour prior. The hyperglucagonemia observed in the LGI/ LGL trial is indicative of a greater depletion of endogenous CHO [48-51] and appears to corroborate suggestions of a lower bioavailability of LGI food-sources during exercise, due to the lower flux of exogenous nutrients from the gut supplementing hepatic glucose production (HGP) and contributing to splanchnic glucose output.

Due to their rapid rate of digestion and absorption, the consumption of HGI foods the hour prior to exercise result in elevated glycemia and insulinemia compared to LGI foodstuff [53-58]. The result is augmented anabolic responses in insulin-sensitive tissues

and the paracrine suppression of glucagon secretion from pancreatic α-cells [39,59,60] (Figure 5.1, postprandial: 60 minutes). Synergistically, the greater insulinemia following a HGI meal [1-3,53-58] combined with contraction mediated pathways [61-66] at exercise onset result in greater peripheral glucose uptake compared to LGI food-sources. Concomitantly the potent insulinogenic suppression of hepatic glucose production (HGP) following HGI food-stuff results in a greater rate of peripheral glucose extraction relative to HGP [18,28] and a transient nadir in blood glucose at the start of exercise [53-58] (Figure 5.1, early exercise: 0-45 minutes). While generally this is not observed following LGI foodstuff [53-58], when LGI food-sources containing significant quantities of non-CHO macronutrients are consumed by trained individuals, transient hypoglycaemia may also be observed, as shown in the present study and elsewhere [27]. This may be due to the potent insulinotropic effects of amino acids (AA) and fatty acids (FA) amplifying glucose-stimulated insulin secretion from β cells [67-69], along with heightened levels of insulin sensitivity in trained individuals [54,70,71]. Originally is was postulated that exercising whilst hypoglycaemic might precipitate the degradation of muscle and liver glycogen [53,58]. Studies that have addressed this concern however have suggested muscle glycogenolysis is unaffected [72,73]. During the mid to late portions of prolonged exercise (Figure 5.1, mid-late exercise > 60 minutes) the slower rate of digestion and absorption of LGI foodstuffs, results in their lower bioavailability compared to HGI meals [5-7]. As exercise duration increases muscle glycogen is depleted and the periphery increasingly extracts and oxidizes blood borne glucose [74,75]. Glucose homeostasis is maintained by a concomitant increase in HGP [76,77]. A greater bioavailability of HGI food-sources facilitates a greater contribution of exogenous CHO, and a lesser role of HGP for

splanchnic glucose output to maintain euglycemia during exercise. Conversely, the lower flux of LGI derived nutrients from the gastrointestinal space and attenuated insulinogenic suppression of HGP, results in a lower contribution of exogenous CHO for splanchnic glucose output and a greater reliance on HGP to maintain blood glucose.

The overall findings of this thesis are supported by the existing literature regarding the physiological effects of the GI and GL both at rest [1,2] and during exercise [5,6]. The LGI or LGL meals generally resulted in lower insulinemia, but higher levels of NEFA availability and glucagonemia. Conversely HGL feedings resulted in the highest insulinemia and insulin/ glucagon ratio but lowest NEFA concentrations. In summary, compared to HGL foods LGI/ LGL feedings evoke lower insulinemia but elevated glucagonemia and NEFA availability at rest and when consumed the hour prior to exercise.

Based on the current literature, possibilities for future research examining the GI and GL at rest and during exercise include:

- Comparison of the metabolic effects of models used to slow the rate of digestion: increased meal frequency versus single bolus ingestion of LGI foodstuff, in order to confirm the validity of previous research.
- Examination of the insulinotropic effects of LGI food sources containing varying amounts of CHO and non-CHO macronutrients both at rest and prior to exercise.
- Comparison of glycemic and insulinemic responses to foods ingested the hour prior to and during exercise between healthy trained, insulin-resistant and diabetic individuals based on the GI and GL.

- Stable isotope studies to direct quantify the rates of digestion and absorption of exogenous nutrients and their bioavailability based on the GI and GL indices using a variety of food sources both at rest and during exercise
- Stable isotope studies using a variety of food sources to directly examine the metabolic availability of exogenous nutrients and their effects on hepatic, skeletal muscle and adipose tissue metabolism based on the GI and GL at rest and prior to/ during exercise.
- Determination of IMP and AMPK responses to CHO containing foods using the GI and GL consumed as pre-exercise meals or feedings.
- Examination of the gluco-regulatory responses based on CHO consumption before exercise using the GI and GL over longer durations and at lower exercise intensities than the present study.

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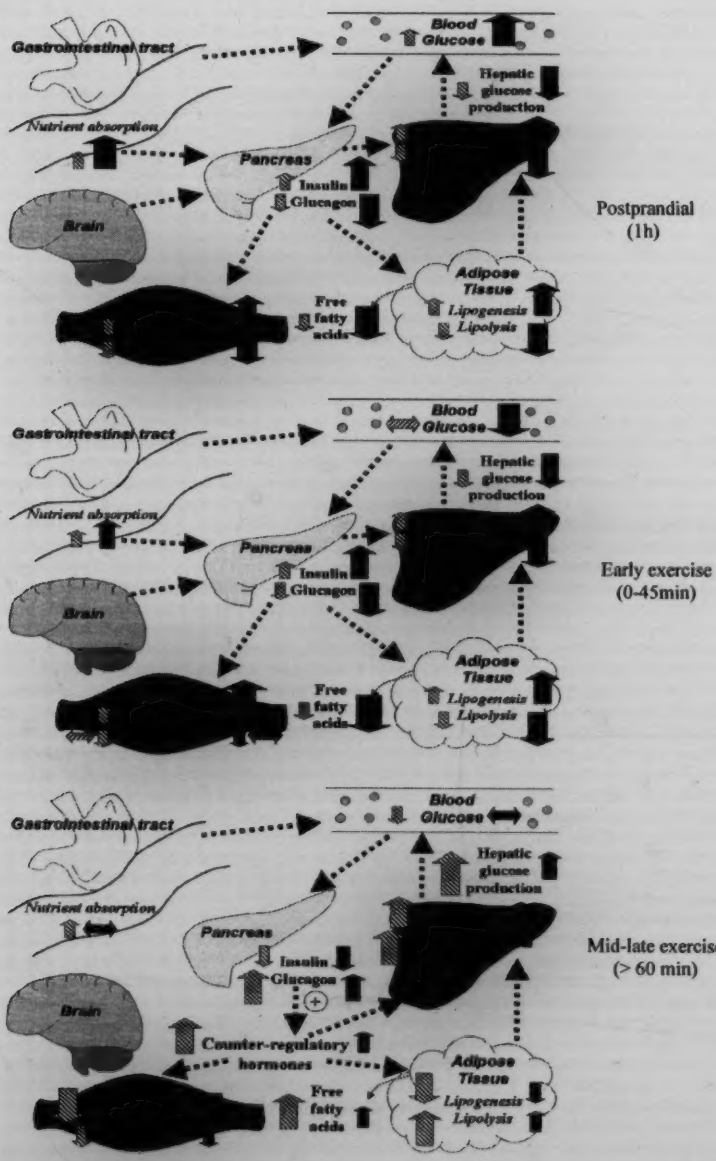


Figure 5.1: 1hr postprandial: rapid absorption of CHO after HGI ⬆ compared to LGI meal ⬇ results in blood glucose spike and high insulin/ glucagon ratio. Early exercise: transient hypoglycemia, continued attenuation of FFA availability following HGI meal. Late exercise: augmented depletion of endogenous CHO stores to maintain euglycemia following a LGI meal due to the lower bioavailability of exogenous nutrients for utilization during exercise. Adapted from Ludwig (2002).

Appendix 1 - Ergoemter ventilatory threshold comparison

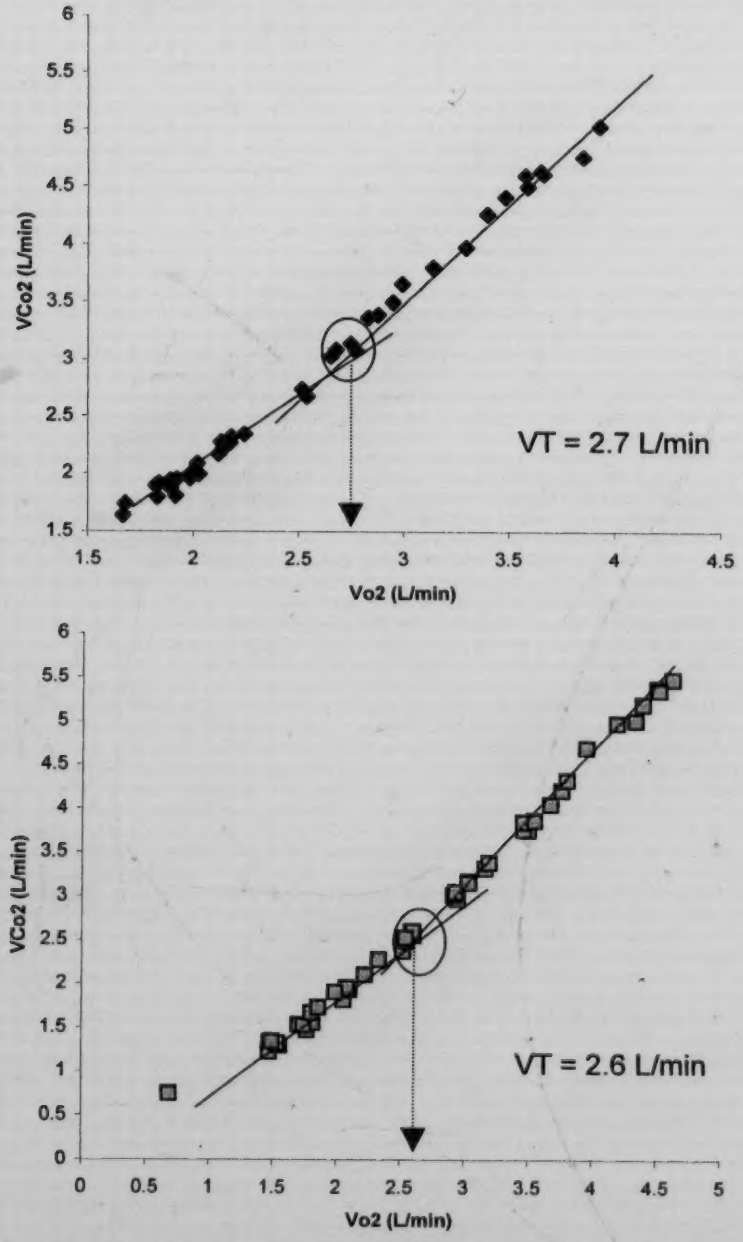


Figure A.1.1. Comparison of Taxc magnetic training devise (top graph) versus Monark cycle ergometer (bottom graph) in individual ventilatory threshold determination using the V-slope method.

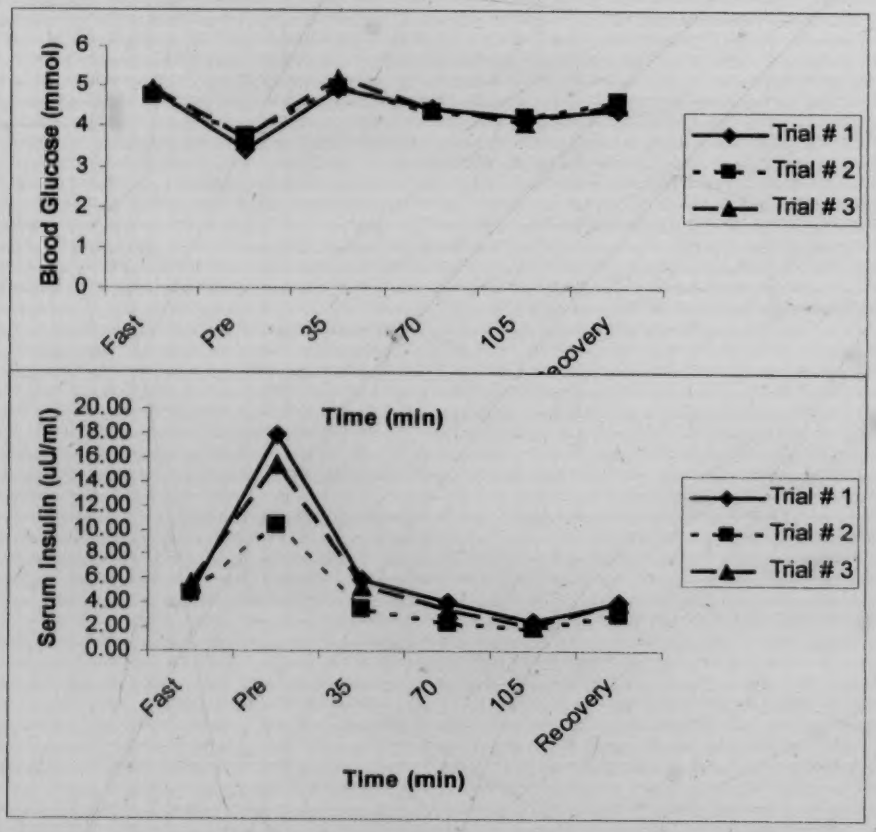
Appendix 2 – Proposal and participant information for study one

A Graduate Student Pilot Project Submitted to the Faculty of Physical Education and Recreation Ethics Committee

Title: ***A pilot study investigating the effect of different nutrition bars on blood glucose and gluco-regulatory hormone response at rest.***

What (exactly) are you doing?

In our previously approved research project (PRE: REB #2005-0503-01), we investigated the blood glucose, lactate and glucoregulatory hormone response to eating 3 different nutrition and exercising. During this project, we discovered an interesting blood hormone response after eating the bars as a part of the planned breakfast prior to exercise. The 2 figures below illustrate this effect.



It was observed that the insulin response was highest after eating the low glycemic index bar (Trial 1 above) compared to the higher glycemic index nutrition bars

conditions (Trials 2 and 3) prior to exercise (note the period of time between Fast and Pre in the graphs above). This occurred despite a similar blood glucose response. However, we only have two blood samples in this time frame and thus we have limited information as to why this response was observed. Since this finding was somewhat unexpected, we felt that it would be prudent to do a separate pilot research study that can investigate this observed phenomena. Therefore we are proposing to assess the blood glucose and glucoregulatory hormone response to eating a standardized amount of 3 different nutrition bars that vary in their glydemic index.

A complete explanation of the glycemic index and its importance in nutrition and during exercise was provided in our previous ethics submission. Briefly, the glycemic index is a scale originally proposed and developed by Jenkins et al. (1981) and ranks foods containing carbohydrate with respect to how they influence blood glucose levels in reference to a particular amount of pure glucose or white bread. This scale can be used to separate foods based on their glycemic response (Foster-Powell et al., 2002). Low GI sources have an advantage for some applications as they allow for a slow release of glucose into the blood and a lower insulin response and vice versa (Walton and Rhodes, 1997). Pre-exercise feeding of a food source with a particular GI is an important consideration for exercise and a pre-exercise spike in blood insulin may be an undesirable response for endurance performance (Earnest et al., 2004; Fabbraio et al., 2000; Thomas et al., 1991). New on the market is a low GI, high carbohydrate food source in a "bar form". This type of nutrition bar has the potential to provide a more favorable blood glycemic response to exercise and be more practical compared to whole meal preparation for active individuals involved in long duration exercise. To our knowledge, there is no research other than our own that has been done to investigate the advantage of this nutritional source on blood glucose and glucoregulatory hormones at rest or during exercise.

Why (what benefits are there to the participants, to society, or to further research? What are you trying to find out?)

The purpose of this pilot study is to determine the blood glucose and glucose regulatory hormone response to a low glycemic index (GI) nutrition bar under resting conditions to characterize the time course of changes in these variables. Based on our previous research, it is hypothesized that the low GI nutrition bar elicits a different time course of blood glucose and glucoregulatory hormone concentrations responses compared to higher GI nutrition bars. This project will provide valuable information on the role of nutrition bars of different GI in providing a more favorable glycemic control under resting conditions, prior to exercise. As part of participation in this study, all subjects will be provided with their personal glucose and hormone levels if desired.

Who are the Participants?

A small sample of 5 – 10 healthy male subjects between the ages of 18 and 35 will be recruited through word of mouth from the University of Alberta. A statistical power calculation for sample size was not performed, since this is a pilot study. All subjects must be healthy and have no knowm conditions that may cause an issue with blood glucose or hormone responses. This will be checked verbally with each subject.

This study will use males only because it is a pilot study with limited funding and the confounding influence of the menstrual cycle on the hormonal measurements.

Where will the study take place?

The testing will be performed in the exercise physiology lab (P340-344). Biochemical analyses will be performed in the exercise biochemistry lab (E443). These labs are within the Faculty of Physical Education and Recreation.

How are you going to do it?

Individuals will be contacted by word of mouth and will be asked to attend an information and orientation meeting. At this meeting, participants will be informed of the purpose and procedure of the study and screened for inclusion criteria verbally and be instructed on how to complete a 1-day dietary record. Once the participant has agreed to participate and completed all forms they will be scheduled for the testing. As well, the subjects will be given a tour of the lab and shown the equipment to be used for the study.

The exercise testing will involve 4 separate visits to the lab: the first is for the orientation meeting. The next three visits will be the 3 nutrition and blood sampling test trials. A registered nurse will take blood samples.

1- Day Dietary Record

Each subject will be give a form that has space for recording the type and amount of food and beverage consumed over one day. They will be instructed on accurate recording technique. This record will be analyzed with a software program (Food Processor II) that will determine the nutritional makeup of the subject's diet. We will modify this diet record if necessary to establish a % breakdown of macro-nutrients of 60% complex carbohydrate, 15% protein and 25% fat. The record or modified record will be returned to the subject and they will be asked to consume the same amounts and types of food on the record, the day before each of the experimental exercise trials.

Blood Glucose and Hormone Response to the Nutrition Bars

The participants will arrive on different days to perform the trials separated by at least 3 days and will be asked not to exercise (day off) 24 hours prior to the morning of each experiment. The subjects will arrive first thing in the morning (between 07:00 and 08:00 am) in the lab after an overnight fast. Upon arrival, body mass is measured and a registered nurse will insert a 22 gauge cathelon, secure it with tape and obtain a fasted blood sample (5 ml) with a syringe. Two ml's of sterile saline will be used to keep the cathelon patent during each experiment. The subjects will then consume the randomly assigned experimental nutrition bar 1 hour before the exercise bouts at an amount equivalent to 1.0 gram of carbohydrate per kg body mass (Tokmakidis et al., 1999). Subjects will be asked to consume 250 ml of water with their meal.

Blood samples will be collected immediately prior to the meal, and every 20 minutes for 2 hours from the cathelon inserted in the arm vein of each subject. Hematocrit, glucose, lactate and glucoregulatory hormones (insulin, glucagon) will be measured in the blood samples. Note that all subjects will be hydrated and normal blood glucose levels obtained (~5 mM) before leaving the lab by providing more water and

food if necessary (blood glucose will be checked immediately in the lab with a portable glucose monitor to ensure this). The total number of blood samples is 7 and amounts to 35 mls that represents no risk to the subject. The 3 different experimental conditions will be performed in a random order and are described below:

Trial A will require the subject to consume the low GI nutrition bar at an amount that will provide 1.0 gram of the carbohydrate source in the bar per kg body mass with 250 ml of water.

Trial B will require the subject to complete consume the carbohydrate source from the high GI nutrition bar at the same amount of 1.0 gram of carbohydrate per kg body mass with 250 ml of water.

Trial C will be use one of the nutrition bars, this time matched for energy intake used in one of the other trials.

This experiment will determine the blood glucose and gluco-regulatory hormone response to eating a low GI nutrition bar (Solo™, GI = 22-28), a moderate to high glycemic index bar (PowerBar™, GI > 58) independently determined by Foster-Powell & Brand-Miller (2002) and a trial that will energy matched the nutrition bars. These two nutrition bars are also very similar in macronutrient proportions (PowerBar™: 9% fat, 18% protein; Solo™: 9% fat, 20% protein).

Additionally, we will ask each subject to rate their hunger and satiety (feeling of fullness) using a visual rating scale that will coincide with the blood samples. This rating scale ranges from 1 to 7 (1 – extremely hungry and 7 being extremely full) and has been validated for use with a variety of types and amounts of meal/food ingestion in humans (Holt et al., 1992; Holt & Brand-Miller, 1994; Holt and Brand-Miller, 1995).

Blood Analysis

The blood collected during the exercise experiments will be analyzed for hematocrit, glucose, lactate, insulin, glucagon and cortisol. Hematocrit will be determined after centrifugation in a 50-ul micro centrifuge tube. Blood glucose & lactate will be measured using spectrophotometric assays routinely performed in our lab. Radio-immunoassays (RIA) will be used to measure the hormone concentrations in the blood. These RIA kits will be purchased from InterMedico, Markham, Ontario. All these assays are also routinely performed in laboratory.

Statistical Analysis

Means and standard deviations will be calculated on dependent variables. If we get 10 subjects, we will use a two-way analysis of variance (ANOVA), with repeated measures to determine significant differences in the blood glucose and hormonal measures between each of the 3 exercise trials over time (7 time points). Any significant F-ratios will be analyzed with a Neuman-Keuls post-hoc test. An alpha of $p<0.05$ will be considered significant.

How long will it take?

Orientation meeting =	30 minutes
Completing diet record at their home =	30 minutes
3-Experitmental trialss =	3*3 hours = 9 hours
Total testing time =	10 hours.

Testing time does not include travel to and from the lab.

What are the qualifications of the research personnel?

All testing will de done under supervision of the principal investigator. Any assistants that may be involved will be graduate students in exercise physiology. A registered nurse will be used for the cathelon procedures.

What are the potential risks of involvement in the study (worst case scenario and likelihood of occurrence) – both to the participants and the researcher?

There is little to no risk to the researchers or research assistants other than general hazards common to working in a laboratory setting. The blood samples are performed under sterile conditions but there is a risk of infection at the site if not properly cared for. This risk will be minimized through sterile procedures, cleanliness and the use of a band-aid. Universal procedures will be followed for all testing procedures, that is, rubber gloves, lab clothing and cleaning all areas with 10% bleach solution that may be in contact with biohazardous material as outlined by the U. of A. Environmental Health and Safety guidelines. The principal investigator has a permit with this office to work with the biohazardous materials that will be encountered in this study

What procedures are in place to deal with potential risks, or what steps have been taken to minimize the possible risks?

All personnel are required to have completed the WHMIS – Biosafety course and have completed a Hepatitis B inoculation. This ensures that all personnel are versed in proper procedures for dealing with biohazardous materials. Only personnel trained in phlebotomy procedures will be allowed to take blood samples from the subjects. The laboratory environment will be maintained in a safe manner and all personnel will follow emergency procedures common to the lab areas.

References available on request.

A pilot study investigating the effect of different nutrition bars on blood glucose and gluco-regulatory hormone response at rest.

Principal Investigator(s): Stephen Cheetham, M.Sc. Student, Faculty of P.E. and Rec. 492-7394.

Co-Investigator(s): Gordon Bell, Ph.D., Faculty of P.E. and Rec. 492-2018.

Vicki Harber, Ph.D., Faculty of P.E. and Rec. 492-1023.

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate, or to withdraw from the study at any time, without consequence, and that your information will be withdrawn at your request?	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your information?	Yes	No

This study was explained to me by: _____

I agree to take part in this study:

_____	_____	_____
Signature of Research Participant	Date	Witness
_____		_____
Printed Name		Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____	_____
Signature of Investigator or Designee	Date

Participant Information

A pilot study investigating the effect of different nutrition bars on blood glucose and gluco-regulatory hormone response at rest.

Principal Investigator: Stephen Cheetham, M.Sc. Student, Faculty of P.E. & Rec. 492-7394.

Co-Investigator: Gordon Bell, Ph.D., Faculty of P.E. & Rec. 492-2018.
Vicki Harber, Ph.D., Faculty of P.E. & Rec. 492-1023.

Dear Participant.

I (Stephen Cheetham) am a graduate student in the Faculty of Physical Education and Recreation under the supervision of Dr. Gordon Bell. I am conducting a pilot study that is researching the effect of eating different nutrition bars on a variety of responses that can be measured in your blood (e.g. glucose, lactate, hematocrit, insulin, glucagon and cortisol). The nutrition bars are commercially available and possibly differ in their effects on blood glucose and hormonal responses.

To be a subject in our study, you must be a healthy male and free of any medical conditions that are known to influence blood glucose or hormone responses (e.g. diabetes). You cannot have any food allergies at all (especially to nuts). You will be asked to attend an orientation meeting in the exercise physiology lab (directions will be provided) during which all the procedures will be completely explained to you and allow us to answer any questions you have related to the study. At this meeting we will also describe and demonstrate all the procedures for you and how to complete a 1-day record of what you eat over 24 hours. You will be asked to take this form home and complete it on your own time. When finished we will ask that you fax or drop this form off at the lab so we can analyze it and modify it if necessary to make sure that your diet is nutritionally sound. Then we will return this form to you and ask you to eat the same foods on the form the day before each of the experimental trials.

At the orientation meeting, we will measure your height and weight and record your age. Then each of the 3 experimental trials will be randomly ordered which means that the order that you do them may or may not be the same for everyone. The day before each trial we will ask you to eat the same meals as indicated above. Then we will ask that you do not eat after 9:00 pm the night before each trial, but you can drink as much water as you require. The next day, we ask that you come to the exercise physiology lab between 7:00 and 8:00 am to begin each experiment. We ask that you do not exercise (ride your bike to the University for example) before you come as we need to take a fasted, rested blood sample. At this time a nurse will put a small sterile tube called a cathelon into a blood vessel in your forearm with a needle. This is similar to what is used if you have had an "IV" in a hospital or when you have donated blood at Blood Services. This means that we only have to "poke" you once with a needle to do the experiments since the needle will be removed in this procedure and only the soft teflon tube remains to take the blood samples from. This tube has a cap on top and is taped to your arm during the test. The nurse will put a small amount of sterile saline (water with some

"electrolytes") into the tube to keep it ready for subsequent blood samples during exercise. We will take 1 blood sample at rest (fasted), and again every 20 minutes for 2 hours. This is 7 samples and totals approximately 35 ml's of blood for each experimental trial which does not present any risk to your health at all.

Depending on the random assignment, you will eventually do each of the 3 experimental conditions described below. You will get at least 3 days off in between the bouts.

During ***trial A***, we will give you one type of nutrition bar, in an amount that will provide you with 1 gram of carbohydrate in the bar calculated per kilogram of your body mass. You will be provided with 250 ml's of water. Blood samples will be taken as described above.

Trial B will provide you with another type of nutrition bar in an amount that is matched to total carbohydrate (1 g/kg) as what was provided to you in trial A. Water intake and blood samples will be conducted in the same way as Trial A.

Ttrial C will be use one of the nutrition bars, this time matched for energy intake used in one of the other trials.

Note that we will provide you with a schedule of all your required visits to the lab and these will be as flexible as possible to suit your personal schedule. The different nutrition bars to be used in this study are Solo™ bar and PowerBar™. We will also ask you to point out a number on a scale displayed on a chart with respect to how hungry you feel before during and after exercise.

Risks: The blood samples are performed with sterile equipment but there is a small risk of infection at the site if not properly cared for. However, sterile procedures, cleanliness and use of a band-aid greatly minimize this risk. A registered nurse using standard procedures will conduct the blood sample procedures using the cathelon.

Qualified personnel under the supervision of Dr. Gordon Bell will administer the testing. Personnel are trained to handle identifiable risks and certifications can be produced upon request. The researchers will continuously watch for adverse symptoms and will stop any procedure if at any time they are concerned about your safety. You can also stop any procedure at any time. Please inform the researcher of any of the above-mentioned symptoms experienced during or after the tests.

Benefits: The major benefit of your participation in this study will be to help the researchers understand the nature of the glucose and hormone response to different nutrition bars that differ in the glycemic index (the ability to maintain blood glucose) and some of the possible physiological mechanisms surrounding it. As a participant you will be provided with a written report of your personal results if you want. If you are interested in the future research outcomes of this study, you may contact one of the researchers for this information as well.

Total Time Commitment:

Orientation meeting	~30 minutes
Completing diet record at home	~30 minutes (total time)

3-experimental trials	~3*3 hours ~ 9 hours
Total testing time =	***~10 hourss***

Testing time does not include travel to and from the lab.

Confidentiality: To ensure confidentiality and anonymity, personal information will be coded and stored in a file cabinet in a locked office to which only the investigators have access. There will be no way to identify individuals in results that may be published in any report or article. Normally, information is retained for a period of 5 years post publication, after which it may be destroyed. The data will hopefully be presented at a research conference and possibly published in a scientific journal.

Freedom to withdraw: For the purpose of the study you are required to participate in all the procedures but you can withdraw at any time without consequence by simply informing one of the investigators verbally, phone call or email. If you decline to continue or withdraw from the study, all information will be removed from the study upon your request. Contacting either Stephen Cheetham or Gordon Bell at anytime during the study can do this.

Additional contacts: If you have concerns about the study and wish to speak with someone who is not involved with this study, please call Dr. Brian Maraj, Associate Dean of Research and Chair of Research Ethics Board, Faculty of Physical Education and Recreation at 492-5910.

Thank you,

_____ _____

_____

Stephen Cheetham **Gordon Bell, Ph.D.** **Vicki Harber, Ph.D.**

Appendix 3 – Proposal and participant information for study two.

A Pilot Project Submitted to the Faculty of Physical Education and Recreation
Ethics Committee

Title: ***A pilot study investigating the effect of nutrition bars of different glycemic index on blood glucose and gluco-regulatory hormone response during cycling.***

What (exactly) are you doing?

During exercise, stores of glucose (from muscle and liver glycogen) and blood sources of glucose are used to support muscular work in a proportion depending on the intensity and duration of exercise and the availability and utilization of other key energy sources such as fatty acids (Chryssanthopolous et al., 2002). It has been established by exercise physiologists and nutritionists that blood glucose maintenance and an optimal glucoregulatory hormone response is necessary to support long duration exercise and that exercise performance can be negatively affected if blood glucose drops (hypoglycaemia). Thus, various nutritional strategies have been developed to support blood glucose, glucoregulatory hormonal response and skeletal muscle metabolism during exercise (Febbraio et al., 2000). This has led to the development of specific athlete meal planning as well as the production of sport drinks and nutrition bars for use before, during and after exercise to aid athletic performance (Baker et al., 1994; Clark, 1997; Takii et al., 2005).

A scale originally proposed and developed by Jenkins et al. (1981) ranks foods containing carbohydrate with respect to how they influence blood glucose levels in reference to a particular amount of pure glucose or white bread. This is termed the glycemic index (GI) and has been used by nutritionists and dieticians to plan meals for a variety of reasons most importantly for someone with diabetes and other metabolic diseases (Jenkins et al., 1981; Foster-Powell et al., 2002). It has also been used by sport nutritionists for planning carbohydrate meals to support exercise demands (Walton and Rhodes, 1997). This scale can be used to separate foods based on their glycemic response (Foster-Powell et al., 2002). Low GI sources have an advantage for some applications as they allow for a slow release of glucose into the blood and a lower insulin response. High GI sources elicit a rapid blood glucose response and a subsequent spike in insulin that can actually cause transient hypoglycemia that may drop below resting levels (Walton and Rhodes, 1997). Pre-exercise feeding of a food source with a high GI source has also been shown to augment carbohydrate utilization during exercise that is an undesirable response for endurance performance (Fabbraio et al., 2000). A pre-exercise low GI source may therefore be more beneficial for long distance exercise as it will allow a slower blood glucose release, lower insulin response, less carbohydrate utilization due to great fat oxidation (Earnest et al., 2004; Fabbraio et al., 2000; Thomas et al., 1991).

The advantage of consuming foods with a low GI for endurance activity is a slower release of glucose into the blood to prolong its availability for the latter stages of a long duration exercise bout and possibly into recovery (Li et al., 2004; Reith et al., 2001). Research has shown that food sources of low GI can provide long-term blood glucose support during exercise more effectively than high GI food sources (Thomas et al. 1991;

Wee et al., 1999). Until recently, however, a low GI strategy for exercise was primarily achieved through "whole meal" planning, careful carbohydrate selection or a particular carbohydrate concentration found in a liquid sport drink. New on the market is a low GI, high carbohydrate food source in a "bar form". This type of nutrition bar has the potential to provide a more favorable blood glycemic response to exercise and be more practical compared to whole meal preparation for active individuals involved in long duration exercise. To our knowledge, there is no research that has been done to investigate the advantage of this nutritional source on blood glucose and glucoregulatory hormones at rest or during exercise.

Why (what benefits are there to the participants, to society, or to further research? What are you trying to find out?)

The purpose of this pilot study is to determine the blood glucose and glucose regulatory hormone response to a low glycemic index (GI) nutrition bar during long duration cycling exercise. It is hypothesized that the low GI nutrition bar will maintain blood glucose and glucoregulatory hormone concentrations better during long duration exercise than a higher GI nutrition bar or energy matched nutrition bar condition. This project will provide valuable information on the role of nutrition bars of different GI in providing a more favorable glycemic control during long duration exercise and hopefully will generate further research for applications such as hiking, trekking, active living and possibly physically active individuals with diabetes mellitus. As part of participation in this study, all subjects will be provided with a free aerobic fitness profile and exercise prescription advice if desired.

Who are the Participants?

A small sample of 5 – 10 healthy male cyclists/triathletes between the ages of 18 and 35 will be recruited through word of mouth from the University of Alberta and by poster in cycling stores and clubs within the city of Edmonton. A statistical power calculation for sample size was not performed, since this is a pilot study. Depending on the preliminary results of this pilot study, the feasibility of performing a future study, with adequate sample size will be assessed. Since it is desirable to have a reasonably heterogeneous level of fitness in the sample, we will actively recruit at least half of the sample from the Edmonton mountain/road/triathlon sporting community who are actively training. All subjects must be free of conditions that may impede their effort and performance during a maximal graded exercise test and this will be assured by completing a Physical Activity Readiness Questionnaire (PAR-Q) along with the informed consent form. We will also ascertain verbally that all subjects have completed at least 105 minutes of continuous cycling in their regular training routine. This study will use males only because it is a pilot study with limited funding and the confounding influence of the menstrual cycle on the hormonal measurements. Consequently, much additional work would be needed to elucidate an appropriate protocol for conducting the proposed study which is beyond the scope of this pilot project.

Where will the study take place?

The exercise testing will be performed in the exercise physiology lab (P340-344). Biochemical analyses will be performed in the exercise biochemistry lab (E443). These labs are within the Faculty of Physical Education and Recreation.

How are you going to do it?

Individuals will be contacted by word of mouth and poster and will be asked to attend an information and orientation meeting. At this meeting, participants will be informed of the purpose and procedure of the study and screened for inclusion criteria verbally and through the Physical Activity Readiness Questionnaire (PAR-Q) and the Healthy Physical Activity Participation Questionnaire (Canadian Physical Activity, Fitness and Lifestyle Approach, 3rd Edition, 2004). They will also be instructed on how to complete a 1-day dietary record. Once the participant has agreed to participate and completed all forms they will be scheduled for the exercise testing. As well, the subjects will be given a tour of the lab and shown the equipment to be used for the study.

The exercise testing will involve 4 separate visits to the lab: the first is for the aerobic fitness test (VT/VO_2max test) at which time they will hand in their 1-day dietary record. The next three visits will be the 3 continuous exercise test trials. A registered nurse will take blood samples before, during and after the submaximal exercise trials.

1- Day Dietary Record

Each subject will be give a form that has space for recording the type and amount of food and beverage consumed over one day. They will be instructed on accurate recording technique. This record will be analyzed with a software program (Food Processor II) that will determine the nutritional makeup of the subject's diet. We will modify this diet record if necessary to establish a % breakdown of macro-nutrients of 60% complex carbohydrate, 15% protein and 25% fat. The record or modified record will be returned to the subject and they will be asked to consume the same amounts and types of food on the record, the day before each of the experimental exercise trials.

Maximal Exercise Test

Each subject will arrive at the lab for his first exercise test in the rested state. This requires that they refrain from any formal exercise 24 hours before the exercise test. All subjects will be instructed to have a light meal of their choice and water ad libitum 2 to 3 hours before the maximal exercise test. The maximal test will require graded, incremental exercise to volitional exhaustion on their own bicycles (using device that allows resistance to be applied to the rear wheel) similar to that outlined by the American College of Sports Medicine (ACSM) guidelines for a graded cycle exercise test (pedal rate = 75 rpm, power out starts at 74 w and is increased by 37 w every two minutes). During the test, each subject will be wearing a headgear and mouthpiece apparatus to collect all the expired air which will be collected and analyzed in a calibrated metabolic measurement system (ParvoMed True Max 2400, Utah). Volitional exhaustion is defined as the point at which the subject cannot continue to exercise due to fatigue. Peak VO_2 will be defined as the highest VO_2 that is recorded during the maximal exercise test that is associated with a respiratory exchange ratio greater than 1.1 and achievement of age-predicted or known maximum heart rate. A 5 minute warm-up and cool-down as well as

stretching will be included. Heart rate will be recorded every minute using a heart rate monitor (Polar USA, CT).

Submaximal Exercise Trials to Determine the Blood Glucose Response to Exercise

The participants will arrive on different days to perform the exercise trials separated by at least 6 days and will be asked not to exercise (day off) 24 hours prior to the morning of each experiment. The subjects will arrive first thing in the morning (between 07:00 and 08:00 am) in the lab after an overnight fast. Upon arrival, body mass is measured and a registered nurse will insert a 22 gauge cathelon, secure it with tape and obtain a fasted blood sample (5 ml) with a syringe. Two ml's of sterile saline will be used to keep the cathelon patent during each experiment. The subjects will then consume the experimental substance 1 hour before the exercise bouts at an amount equivalent to 1.0 gram of carbohydrate per kg body mass (Tokmakidis et al., 1999). Each participant will complete 3 randomly assigned, standardized 1 hour and forty five minute cycling exercise tests on their own racing bikes that will be attached to a stationary, magnetic training device. The intensity will be standardized at the heart rate that elicited the intensity equivalent to 10% below the individual VT (determined from the aerobic fitness test). This intensity and potential distance covered is similar to that reported by Earnest et al. (2004) that influenced glycemic control. We have also confirmed this in our lab. Subjects will be asked to consume 250 ml of water, twice during the hour before the exercise test and 200 ml every 20 minutes during exercise (American College of Sports Medicine exercise hydration guidelines) during each experimental trial. Metabolic measurements will be taken over a 5-minute period after 35, 70 and 105 minutes (end) of the exercise test to measure oxygen consumption and respiratory exchange ratio that can be used to estimate the percent carbohydrate and fat utilized during exercise. Heart rate will also be monitored to ensure the intensity of exercise is adhered to.

Blood samples will be collected immediately prior to exercise, after 35, 70 and 105 minutes (end) of exercise; and, after 1 hour of recovery (5 blood samples of 5 ml) from the cathelon inserted in the arm vein of each subject. Hematocrit, glucose, lactate, free fatty acids and the glucoregulatory hormones insulin, glucagon and cortisol will be measured in the blood samples. Body mass will be measured after exercise and recovery to monitor any body water losses due to possible dehydration. Note that all subjects will be hydrated and normal blood glucose levels obtained (~5 mM) before leaving the lab by providing more water and food if necessary (blood glucose will be checked immediately in the lab with a portable glucose monitor to ensure this). The total number of blood samples is 6 and amounts to 30 ml that represents no risk to the subject. The 3 different experimental conditions will be performed in a random order and are described below:

Trial A will require the subject to complete the 1hour, 45-minute cycling exercise test after consuming the low GI nutrition bar at an amount that will provide 1.0 gram of the carbohydrate source in the bar per kg body mass, 1 hour before the exercise bout with ~250 ml of water (fluid intake will be matched to Trial A). This will be calculated based on the known

amount listed by the manufacturer. In addition 250 ml of water will be consumed every 20 minutes during exercise and recovery.

Trial B will require the subject to complete 1hour, 45-minute cycling exercise test after consuming the carbohydrate source from the high GI nutrition bar at the same amount of 1.0 gram of carbohydrate per kg body mass, 1 hour before the exercise bout in ~250 ml of water (fluid intake will be matched to Trial A). In addition 250 ml of water will be consumed every 20 minutes during exercise and recovery.

Trial C will be use one of the nutrition bars, this time matched for energy intake used in one of the other trials.

This experiment will determine the blood glucose and gluco-regulatory hormone response to a low GI nutrition bar (Solo™, GI = 22-28), a moderate to high glycemic index bar (PowerBar™, GI > 58) independently determined by Foster-Powell & Brand-Miller (2002) and a trial that will energy matched the nutrition bars. These two nutrition bars are also very similar in macronutrient proportions (PowerBar™: 9% fat, 18% protein; Solo™: 9% fat, 20% protein).

Additionally, we will ask each subject to rate their hunger and satiety (feeling of fullness) using a rating scale that will coincide with the blood samples (fasted, pre-exercise, after 35, 70 and 105 minutes and after 1 hour of recovery). This rating scale ranges from 1 to 7 (1 – extremely hungry and 7 being extremely full) and has been validated for use with a variety of types and amounts of meal/food ingestion in humans (Holt et al., 1992; Holt & Brand-Miller, 1994; Holt and Brand-Miller, 1995).

Blood Analysis

The blood collected during the exercise experiments will be analyzed for hematocrit, glucose, lactate, fatty acids, insulin, glucagon and cortisol. Hematocrit will be determined after centrifugation in a 50-ul micro centrifuge tube. Blood glucose, lactate and fatty acids will be measured using spectrophotometric assays routinely performed in our lab. Radio-immunoassays (RIA) will be used to measure the hormone concentrations in the blood. These RIA kits will be purchased from InterMedico, Markham, Ontario. All these assays are also routinely performed in laboratory.

Statistical Analysis

Means and standard deviations will be calculated on dependent variables. If we get 10 subjects, we will use a two-way analysis of variance (ANOVA), with repeated measures to determine significant differences in the blood glucose and hormonal measures between each of the 3 exercise trials over time (6 time points) in both the resting and exercise experiments. Any significant F-ratios will be analyzed with a Neuman-Keuls post-hoc test. An alpha of $p<0.05$ will be considered significant.

How long will it take?

Orientation meeting =	30 minutes
Completing diet record at their home =	30 minutes
Maximal exercise test =	45 minutes
3-submaximal exercise trials =	3*4 hours = 12 hours
Total testing time =	13 hours and 45 minutes

Testing time does not include travel to and from, changing and showering.

What are the qualifications of the research personnel?

All testing will de done under supervision of the principal investigator. Any assistants that may be involved will be graduate students in exercise physiology. There will be someone certified in CPR during each testing session. A registered nurse will be used for the cathelon procedures.

What are the potential risks of involvement in the study (worst case scenario and likelihood of occurrence) – both to the participants and the researcher?

There is little to no risk to the researchers or research assistants other than general hazards common to working in a laboratory setting. The peak VO_2 exercise test requires maximal physical effort and motivation. The blood samples are performed under sterile conditions but there is a risk of infection at the site if not properly cared for. This risk will be minimized through sterile procedures, cleanliness and the use of a band-aid. Universal procedures will be followed for all testing procedures, that is, rubber gloves, lab clothing and cleaning all areas with 10% bleach solution that may be in contact with biohazardous material as outlined by the U. of A. Environmental Health and Safety guidelines. The principal investigator has a permit with this office to work with the biohazardous materials that will be encountered in this study

What procedures are in place to deal with potential risks, or what steps have been taken to minimize the possible risks?

All personnel are required to have completed the WHMIS – Biosafety course and have completed a Hepatitis B inoculation. This ensures that all personnel are versed in proper procedures for dealing with biohazardous materials. Only personnel trained in phlebotomy procedures will be allowed to take blood samples from the subjects. The laboratory environment will be maintained in a safe manner and all personnel will follow emergency procedures common to the lab areas.

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Informed Consent

A pilot study investigating the effect of nutrition bars of different glycemic index on blood glucose and gluco-regulatory hormone response during cycling.

Principal Investigator(s): Stephen Cheetham, M.Sc. Student, Faculty of P.E. and Rec. 492-7394

Co-Investigator(s): Gordon Bell, Ph.D., Faculty of P.E. and Rec. 492-2018

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate, or to withdraw from the study at any time, without consequence, and that your information will be withdrawn at your request?	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your information?	Yes	No

This study was explained to me by: _____

I agree to take part in this study:

_____	_____	_____
Signature of Research Participant	Date	Witness
_____		_____
Printed Name		Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____	_____
Signature of Investigator or Designee	Date

Participant Information

A pilot study investigating the effect of nutrition bars of different glycemic index on blood glucose and gluco-regulatory hormone response during cycling.

Principal Investigator: Stephen Cheetham, M.Sc. Student, Faculty of P.E. & Rec. 492-7394.
Co-Investigator: Gordon Bell, Ph.D., Faculty of P.E. & Rec. 492-2018.

Dear Participant.

I (Stephen Cheetham) am a graduate student in the Faculty of Physical Education and Recreation under the supervision of Dr. Gordon Bell. I am conducting a pilot study that is researching the effect of 2 different nutrition bars during 3 separate exercise trials on a variety of responses that can be measured in your blood (e.g. glucose, lactate, free fatty acids, hematocrit, insulin, glucagon and cortisol) before, during and after long duration cycling exercise. The 2 nutrition bars are commercially available and possibly differ in their ability to maintain blood glucose during exercise. The type of exercise we will ask you to do to determine the effectiveness of the different nutrition bars is 1 hour and 45 minutes of cycling in the lab at a moderate intensity.

To be a subject in our study, you must be a healthy male and free of any medical conditions that would limit you from performing maximal exercise. You cannot have any food allergies at all (especially to nuts). We will require that you complete a physical activity readiness questionnaire (PAR-Q form). We also want subjects in our study that are involved in road cycling, mountain biking or triathloning so we will request that you complete a physical activity participation form to determine this. You will be asked to attend an orientation meeting in the exercise physiology lab (directions will be provided) during which all the procedures will be completely explained to you and allow us to answer any questions you have related to the study. At this meeting we will also describe and demonstrate all the exercise procedures for you and how to complete a 1-day record of what you eat over 24 hours. You will be asked to take this form home and complete it on your own time. When finished we will ask that you fax or drop this form off at the lab so we can analyze it and modify it if necessary to make sure that your diet is nutritionally sound. Then we will return this form to you and ask you to eat the same foods on the form the day before each of the long distance, cycling exercise trials described later.

The first exercise test is a maximal aerobic fitness test and will be on a different day. Before the exercise test, we will measure your height and weight and record your age. Then you will get ready to perform an exercise test (on your own bicycle on a "mag training device") that gradually gets harder and harder every 2 minutes until exhaustion (until you indicate that you cannot continue by stopping the test) to determine your peak aerobic fitness (peak VO_2) and your ventilatory threshold (VT). The information from this test will also be used to determine the intensity that you will perform the exercise trials. The actual test usually lasts for about 15 minutes, with an additional 5 to 10 minutes of warm-up and cool-down exercise before and after. During the test, you will be

required to wear a nose clip and you will be breathing into a mouthpiece attached to a special breathing apparatus so that all the air you breathe out is collected into a machine that will determine a variety of things such as your oxygen consumption. Heart rate is monitored continuously with a heart rate monitor that is strapped around your chest. We also ask that you do not do any formal exercise (e.g. training) the day before any of your exercise tests. As well, we ask that you have a light meal of your choice and drink 500 ml's of water 2 to 3 hours before this exercise test. After 2 to 4 days, we will set up another time for the first exercise trial.

There will be 3 different exercise trials on 3 different days and over 2-3 different weeks. We would like to coordinate this so it fits into your regular training program for your convenience. Each of the 3 exercise trials will be randomly ordered which means that the order that you do them may or may not be the same for everyone. Each exercise test will be performed at the same moderate intensity (equal to 10% below your VT) determined during your aerobic fitness test and for the same length of time (1 hour and 45 minutes of cycling). Note that these exercise trials are not as fast as you can go in 1 hour and 45 minutes (not a race!), but are controlled intensity cycling bouts and could be described to be similar to a training session. You will be able to use your own bike to do these exercise trials in the lab because we will use stationary devices ("mag trainers") that you can set your bikes on. The day before each exercise trial we will ask you to eat the same meals as indicated above. Then we will ask that you do not eat after 9:00 pm the night before each trial, but you can drink as much water as you require. The next day, we ask that you come to the exercise physiology lab between 7:30 and 8:30 am to begin each experiment. We ask that you do not exercise (ride your bike to the University for example) before you come as we need to take a fasted, rested blood sample. At this time a nurse will put a small sterile tube called a cathelon into a blood vessel in your forearm with a needle. This is similar to what is used if you have had an "IV" in a hospital or when you have donated blood at Blood Services. This means that we only have to "poke" you once with a needle to do the experiments since the needle will be removed in this procedure and only the soft teflon tube remains to take the blood samples from. This tube has a cap on top and is taped to your arm during the test. The nurse will put a small amount of sterile saline (water with some "electrolytes") into the tube to keep it ready for subsequent blood samples during exercise. We will take 1 blood sample at rest (fasted), 1 immediately before exercise (after eating), again after 35, 70 and at the end of exercise, and 1 more an hour after recovery. This is 6 samples and totals approximately 30 ml's of blood for each exercise trial experiment which does not present any risk to your health or exercise performance at all.

Depending on the random assignment, you will eventually do each of the 3 experimental conditions described below. Remember each condition is cycling for 1 hour and 45 minutes at the same intensity (10% below your VT) using a target heart rate range. You will get a week off in between the bouts.

During ***exercise trial A***, we will give you one type of nutrition bar, 1 hour before your exercise in an amount that will provide you with 1 gram of carbohydrate in the bar calculated per kilogram of your body mass. You will be provided with water during the exercise trial. The exercise will begin 1 hour after you eat the bar. Blood samples will be taken as described above.

Exercise trial B will provide you with another type of nutrition bar in an amount that is matched to total carbohydrate (1 g/kg) as what was provided to you in trial A. You will be provided with water during the exercise trial. The exercise will begin 1 hour after you eat the bar. Blood samples will be taken as described above.

Exercise trial C will be use one of the nutrition bars, this time matched for energy intake used in one of the other trials.

Note that we will provide you with a schedule of all your required visits to the lab and these will be as flexible as possible to suit your personal schedule. The two different nutrition bars to be used in this study are Solo™ bar and PowerBar™. We will also ask you to point out a number on a scale displayed on a chart with respect to how hungry you feel before during and after exercise.

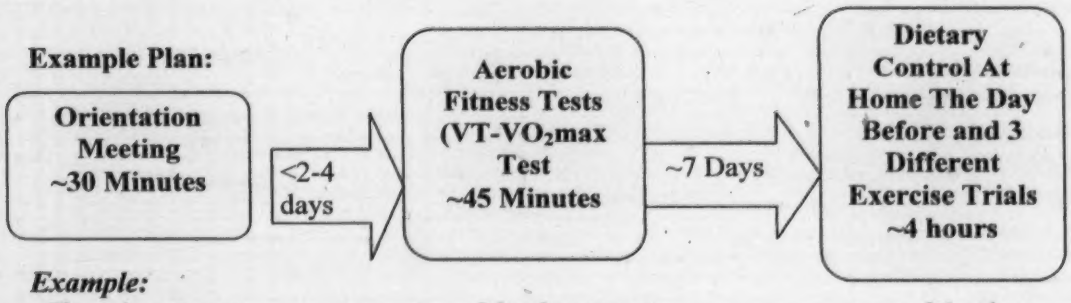
Risks: The maximal (peak VO_2) exercise test requires maximal effort to go to exhaustion and/or perform to each person's maximal capacity. With this type of exercise there may be some health risk. During and after the tests it is possible to experience symptoms such as abnormal blood pressure, fainting, lightheadedness, muscle cramps or strain, nausea, and in very rare cases (0.5 per 10,000 in testing facilities such as exercise laboratories, hospitals and physicians offices), heart rhythm disturbances or heart attack for the VO_2max Test. While serious health risk to you is highly unlikely, these risks must be acknowledged, and you willingly assume the risks associated with very hard exercise. The submaximal test is of moderate intensity but of long duration and is considered to be less risk.

The blood samples are performed with sterile equipment but there is a small risk of infection at the site if not properly cared for. However, sterile procedures, cleanliness and use of a band-aid greatly minimize this risk. A registered nurse using standard procedures will conduct the blood sample procedures using the cathelon.

Qualified personnel under the supervision of Dr. Gordon Bell will administer the exercise testing. Personnel are trained to handle identifiable risks and emergencies and have certification in CPR. Certifications can be produced upon request. The researchers will continuously watch for adverse symptoms and will stop the test if at any time they are concerned about your safety. You can also stop the test at any time. Please inform the researcher of any of the above- mentioned symptoms experienced during or after the tests.

Benefits: The major benefit of your participation in this study will be to help the researchers understand the nature of the glucose response to different nutrition bars during long duration cycling exercise and some of the possible physiological mechanisms surrounding it. As a participant you will be provided with a written report of your personal aerobic fitness information and an exercise training prescription if you want. If you are interested in the future research outcomes of this study, you may contact one of the researchers for this information as well.

Example Plan:


Orientation Meeting ~30 Minutes
<2-4 days
Aerobic Fitness Tests (VT-VO_2max Test ~45 Minutes
~7 Days
Dietary Control At Home The Day Before and 3 Different Exercise Trials ~4 hours

Example:

Thursday (Day 1) → Monday (Day 4) → Monday (1 week later)

Total Time Commitment:

Orientation meeting	~30 minutes
Completing diet record at home	~30 minutes (total time)
Maximal exercise test	~45 minutes
3-submaximal exercise trials	~3*4 hours ~ 12 hours
Total testing time =	***~13 hours and 45 minutes***

Testing time does not include travel to and from, changing and showering.

Confidentiality: To ensure confidentiality and anonymity, personal information will be coded and stored in a file cabinet in a locked office to which only the investigators have access. There will be no way to identify individuals in results that may be published in any report or article. Normally, information is retained for a period of 5 years post publication, after which it may be destroyed. The data will hopefully be presented at a research conference and possibly published in a scientific journal.

Freedom to withdraw: For the purpose of the study you are required to participate in all the procedures but you can withdraw at any time without consequence by simply informing one of the investigators verbally, phone call or email. If you decline to continue or withdraw from the study, all information will be removed from the study upon your request. Contacting either Stephen Cheetham or Gordon Bell at anytime during the study can do this.

Additional contacts: If you have concerns about the study and wish to speak with someone who is not involved with this study, please call Dr. Brian Maraj, Associate Dean of Research and Chair of Research Ethics Board, Faculty of Physical Education and Recreation at 492-5910.

Thank you,

Stephen Cheetham **Gordon Bell, Ph.D.**

Appendix 4 – Data collection sheet – study one

Subject Name	
Trial #	
Subject Weight (kg)	
Time taken to consume meal (min: sec)	

	Hematocrit	Palatability	Satiety
0 min			
MEAL			
30 min			
60 min			
90 min			
120 min			
150 min			

Appendix 5 – Data collection sheet study two.

Subject Name		Height		Pre-Weight		Post-Weight		Age	

	GLUCOSE	LACTATE	HEMATOCRIT
FASTING			

	60 MIN	30 MIN
PALATABILITY		

EXERCISE								
	HEART RATE	VO^2 (L/min)	RER	RPE	SATIETY	GLUCOSE	LACTATE	HEMATOCRIT
0-5 min								
5-10 min								
10-15 min								
15-20 min								
20-25 min								
25-30 min								
30-35 min								
35-40 min								
40-45 min								
45-50 min								
50-55 min								
55-60 min								
60-65 min								
65-70 min								
70-75 min								
75-80 min								
80-85 min								
85-90 min								
90-95 min								
95-100 min								
100-105 min								

	GLUCOSE	LACTATE	HEMATOCRIT
+ 60 MIN			

	60 MIN +	30 MIN +
SATIETY		

POWER OUTPUT (WATTS)	

SIGNAL DISPLAY	Pre	Post
O^2		
CO^2		

Appendix 6 – Additional tables for study one.

Table A.6: Change in hematocrit (%) over time for study one. Means ± S.D.

	LGI/ LGL (1g CHO/ kg)	HGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
0 min (fasting)	44 ± 2.7	44 ± 3.1	44 ± 2.8
150 min (postprandial)	43 ± 2.9	43 ± 3.2	44 ± 3.4
Δ (% change)	1 ± 1.4	1 ± 1.6	0 ± 1.5

Appendix 7 - % change in variables based on trial during study one.

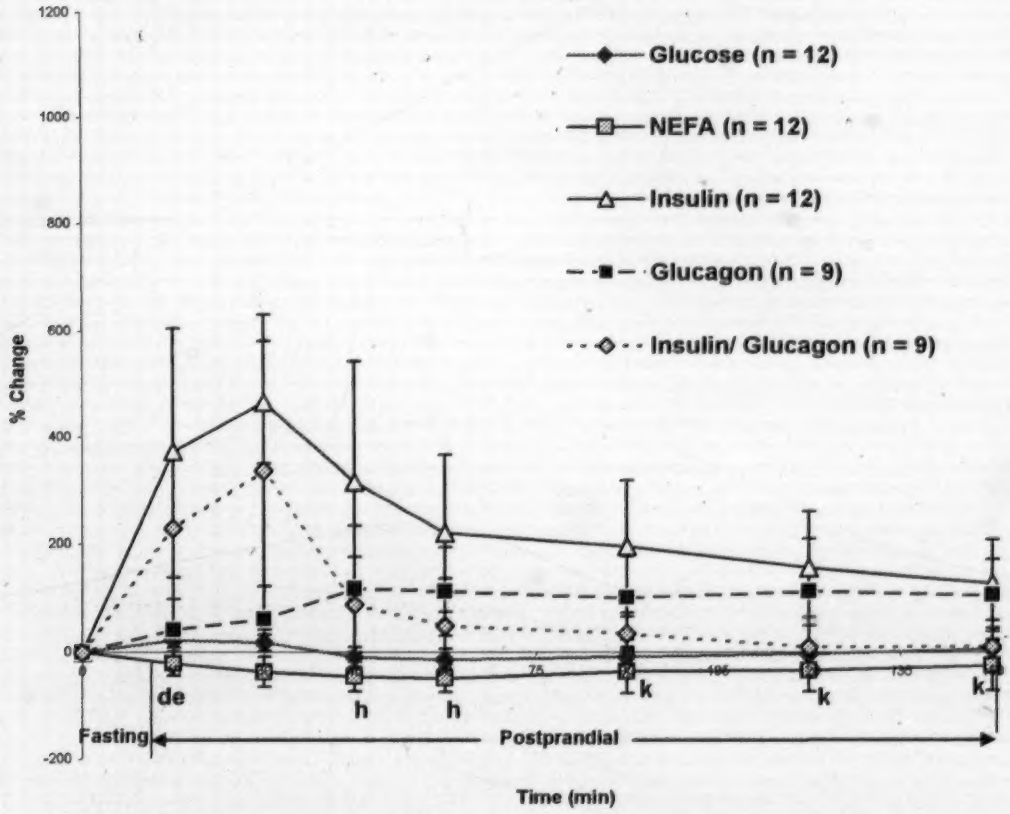


Figure A7.1: Percent change in blood variables throughout the course of LGI/ LGL (1g CHO/ kg). Values are means ± S.D. d = significantly different from 45 min; e = significantly different from 60 min; h = significantly different from 150 min; k = significantly different from MGI/ HGL trial. $P < 0.05$.

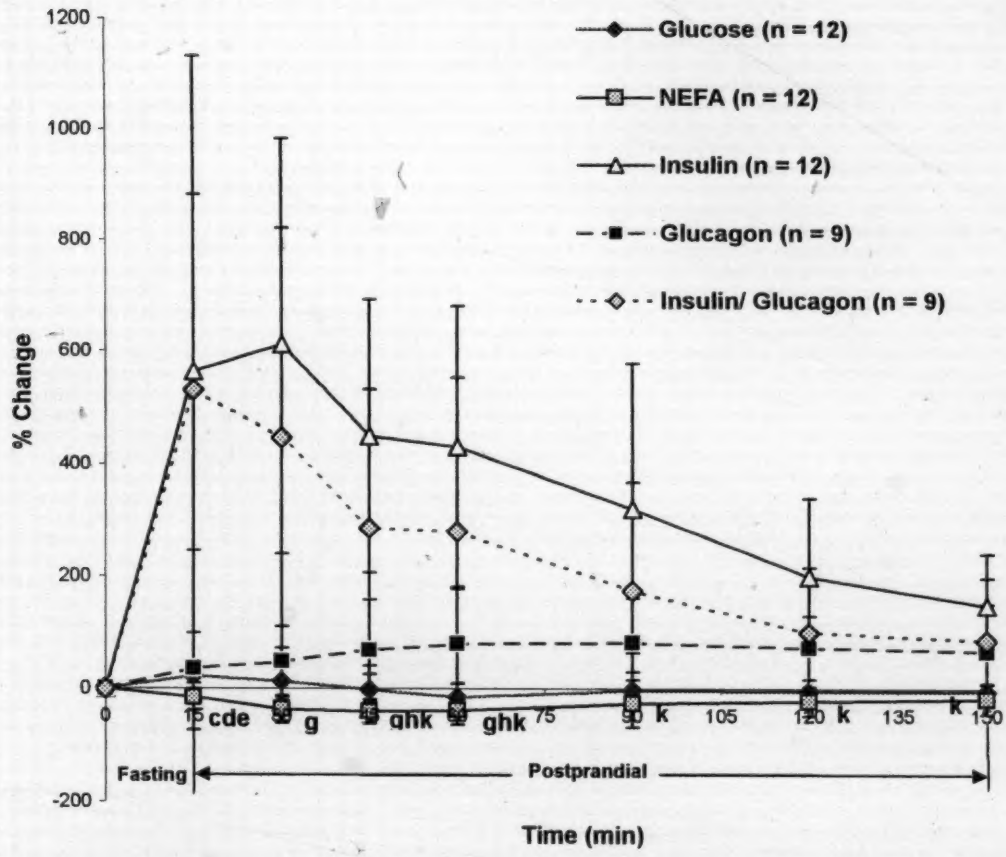


Figure A7.2: Percent change in blood variables throughout the course of HGI/ MGL (1g CHO/ kg). Values are means ± S.D. c = significantly different from 30 min; d = significantly different from 45 min; e = significantly different from 60 min; g = significantly different from 120 min; h = significantly different from 150 min; k = significantly different from MGI/ HGL trial. $P < 0.05$.

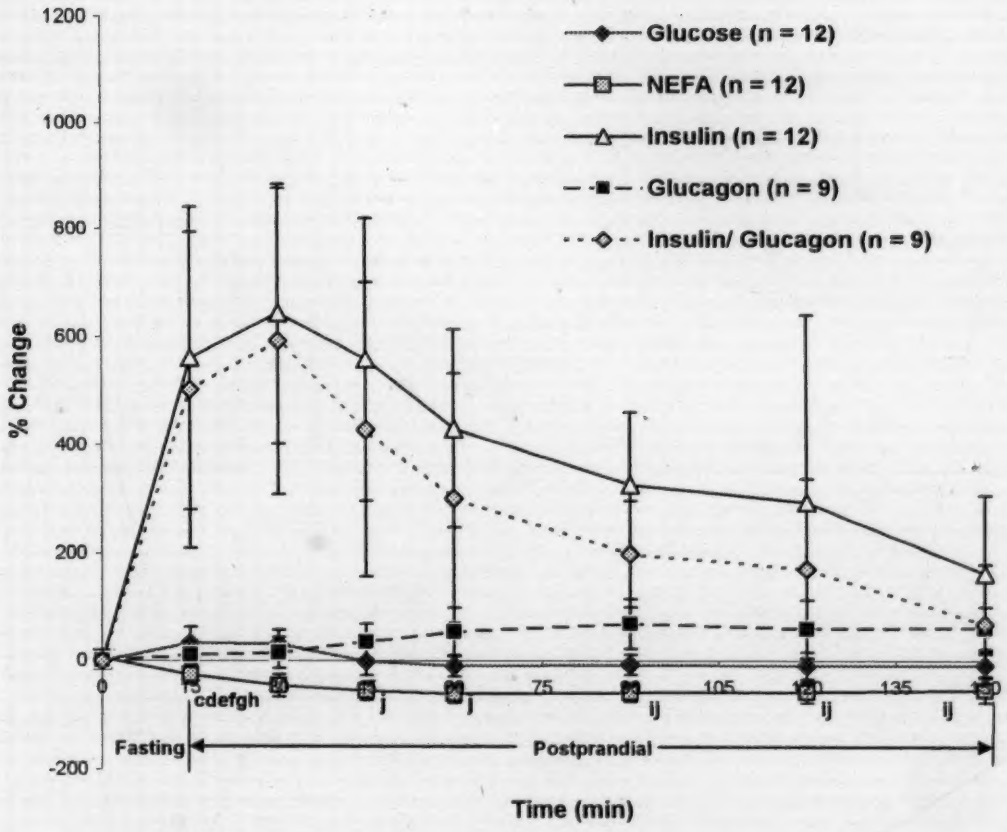


Figure A.7.3: Percent change in blood variables throughout the course of MGI/ HGL (isocaloric). Values are means ± S.D; c = significantly different from 30 min; d = significantly different from 45 min; e = significantly different from 60 min; f= significantly different from 90 min; g = signficantly different from 120 min; h = significantly different from 150 min; i = significantly different from LGI/ LGL; j = significantly different from HGI/ MGLP < 0.05.

Appendix 8 – Additional tables for study two.

Table A.8.1. Maximal exercise test data for study two. Values are means ± S.D.

	Mean ± S.D.
$VO_{2\ peak}$ (L/min)	4.31 ± 0.63
$VO_{2\ peak}$ (ml/kg/min)	56.52 ± 10.36
HR_{peak} (bpm)	183.4 ± 7.27
Peak Power Output (Watts)	430.0 ± 60.0
Ventilatory Threshold (L/min)	3.39 ± 0.49
Ventilatory Threshold (% Vo2 max)	79.40 ± 2.07
HR @ Ventilatory Threshold (bpm)	160.6 ± 6.07
Power Output @ Ventilatory Threshold (Watts)	250.0 ± 56.12

Table A.8.2. Ambient conditions during 105 minutes of cycling following the three test meals for study two. Values are means ± S.D..

	LGI/ LGL (1g CHO/ kg	MGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
Barometric Pressure (mm Hg)	703 ± 2.9	707.1 ± 3.5	704.5 ± 5.7
Temperature (deg C)	21.5 ± 0.7	21.5 ± 0.9	21.3 ± 0.7
Humidity (%)	29.5 ± 8.0	29.4 ± 7.7	31.1 ± 13.0

Table A.8.3. Body water loss during 105 minutes of cycling following the three test meals for study two. Values are means ± S.D..

	LGI/ LGL (1g CHO/ kg)	MGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
Pre exercise body mass (kg)	77.66 ± 3.5	76.94 ± 4.0	77.46 ± 3.6
Post exercise body mass (kg)	77.04 ± 3.7	76.3 ± 4.1	76.66 ± 3.6
Δ change (kg)	-0.62 ± 0.3	-0.64 ± 1.0	-0.8 ± 0.5

Table A.8.4. Physiological data summary during 105 minutes of cycling following the three test meals for study two. Values are means ± S.D. n = 5 subjects.

	LGI/ LGL (1g CHO/ kg)	MGI/ MGL (1g CHO/ kg)	MGI/ HGL (isocaloric)
PO (Watts)	207.61 ± 30.17	207.61 ± 30.17	207.61 ± 30.17
% of PPO	47.71 ± 4.57	47.71 ± 4.57	47.71 ± 4.57
VO_2 (L/min)	3.06 ± 0.35	3.01 ± 0.42	3.02 ± 0.45
% below VT	8.10 ± 4.71	9.44 ± 5.01	8.92 ± 5.57
RER	0.93 ± 0.02	0.93 ± 0.03	0.93 ± 0.03
Carbohydrate Oxidation (g)	301.75 ± 51.28	307.11 ± 56.80	307.55 ± 50.22
Energy Expenditure (kcal)	1591.53 ± 188.56	1574.88 ± 204.89	1571.15 ± 225.25
Heart Rate (bpm)	149 ± 10	147 ± 11	147 ± 13

Table A.8.5. Change in hematocrit (%) over time following the three test meals for study two. Values are means ± S.D.. -80 min = fasting sample; 170 min = 60 min post-exercise sample.

	LGI/ LGL (1g CHO/ kg)	MGI/ LGL (1g CHO/kg)	MGI/ MGL (isocaloric)
-80 min (fasting)	44 ± 3.4	45 ± 2.8	44 ± 3.3
170 min (60 min post-exercise)	45 ± 2.6	45 ± 2.9	45 ± 1.8
Δ	1 ± 0.7	0 ± 0.7	1 ± 1.7

Table A.8.6. Change in blood lactate (mmol) during 105 minutes of cycling following the three test meals for study two. Values are means ± S.D. -80 min = fasting sample; -10 min = pre-exercise sample; 35, 70 & 105 min = exercise samples; 170 min = 60 min post-exercise sample. b = significantly different from -10 min; h = significantly different from MGI/ LGL (1g CHO/ kg). $P < 0.05$.

Time (min)	LGI/ LGL (1g CHO/ kg)	MGI/ LGL (1g CHO/kg)	MGI/ MGL (isocaloric)
-80	1.16 ± 0.35	1.12 ± 0.22	1.14 ± 0.27
-10	1.78 ± 0.8	1.92 ± 0.6	1.76 ± 0.6
35	3.46 ± 1.8[b]	3.52 ± 1.6[b]	4.04 ± 3.3[b]
70	2.42 ± 1.2	2.74 ± 1.2	3.24 ± 1.8
105	2.22 ± 0.9[h]	2.76 ± 0.8	2.74 ± 1.2
170	1.62 ± 0.63	1.38 ± 0.29	1.8 ± 0.72

Appendix 9 - additional graphs study one.

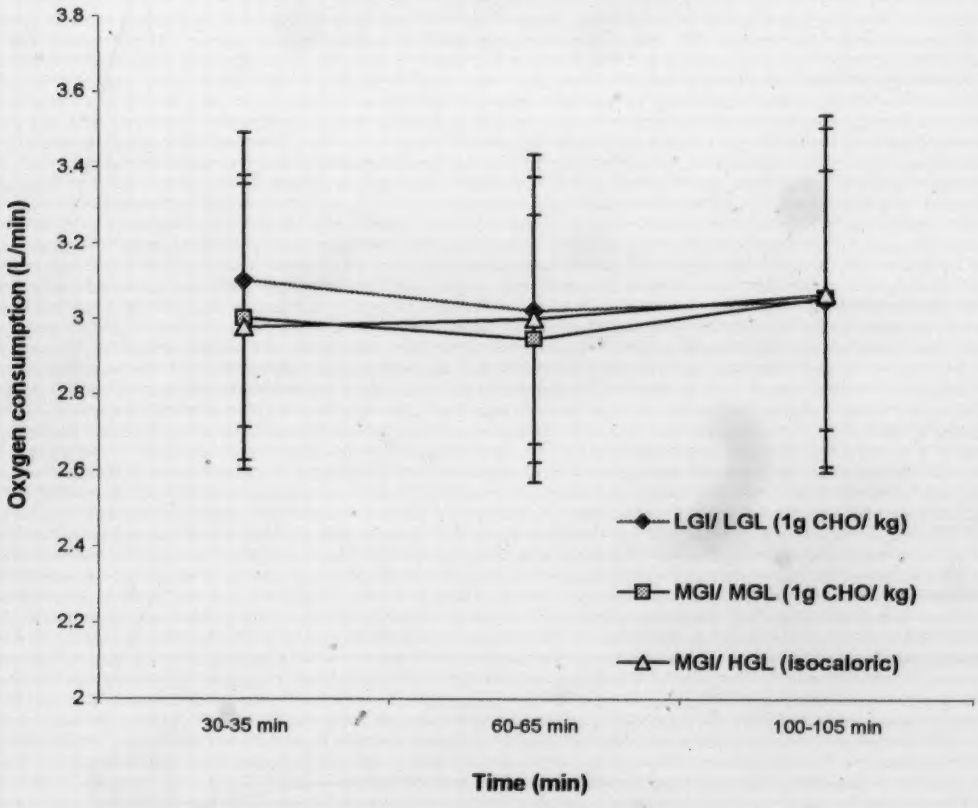


Figure A.9.1: Oxygen consumption during 105 minutes of cycling following the three test meals. Values are means ± S.D. $P < 0.05$.

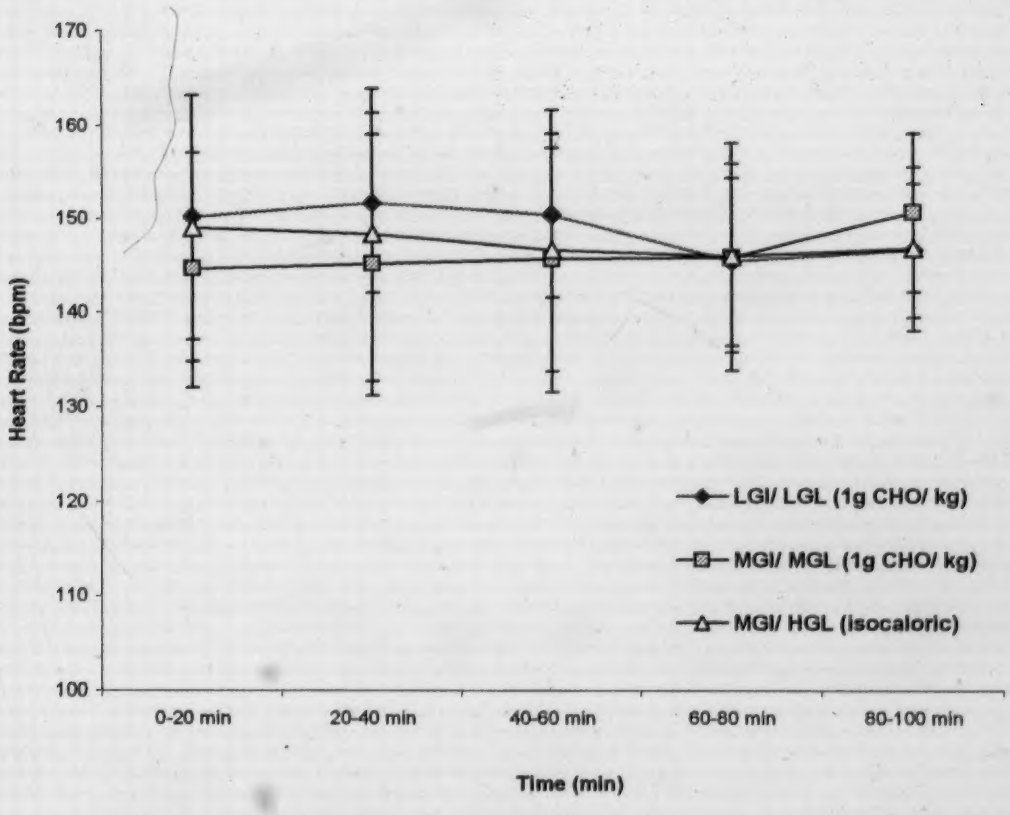


Figure A.9.2: Heart rates (bpm) during 105 minutes of cycling following the three test meals. Values are means ± S.D. $P < 0.05$.

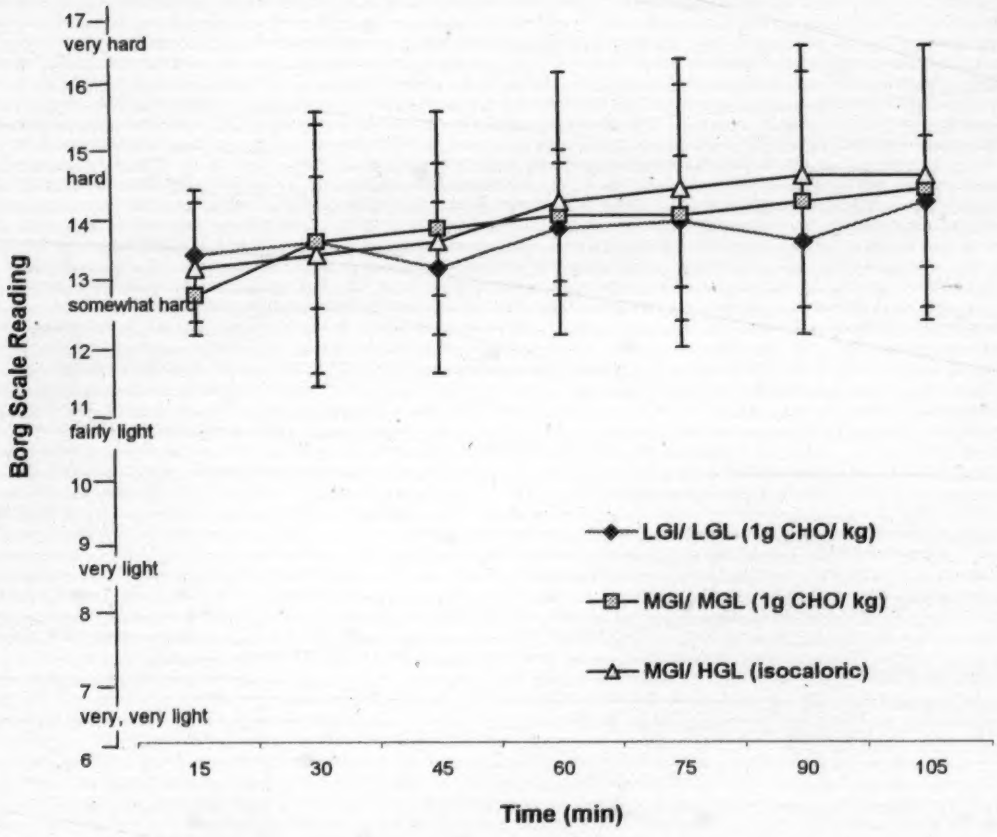


Figure A.9.3: Ratings of percieved exertion during 105 minutes of cycling following the three test meals. Values are means ± S.D. $P < 0.05$.

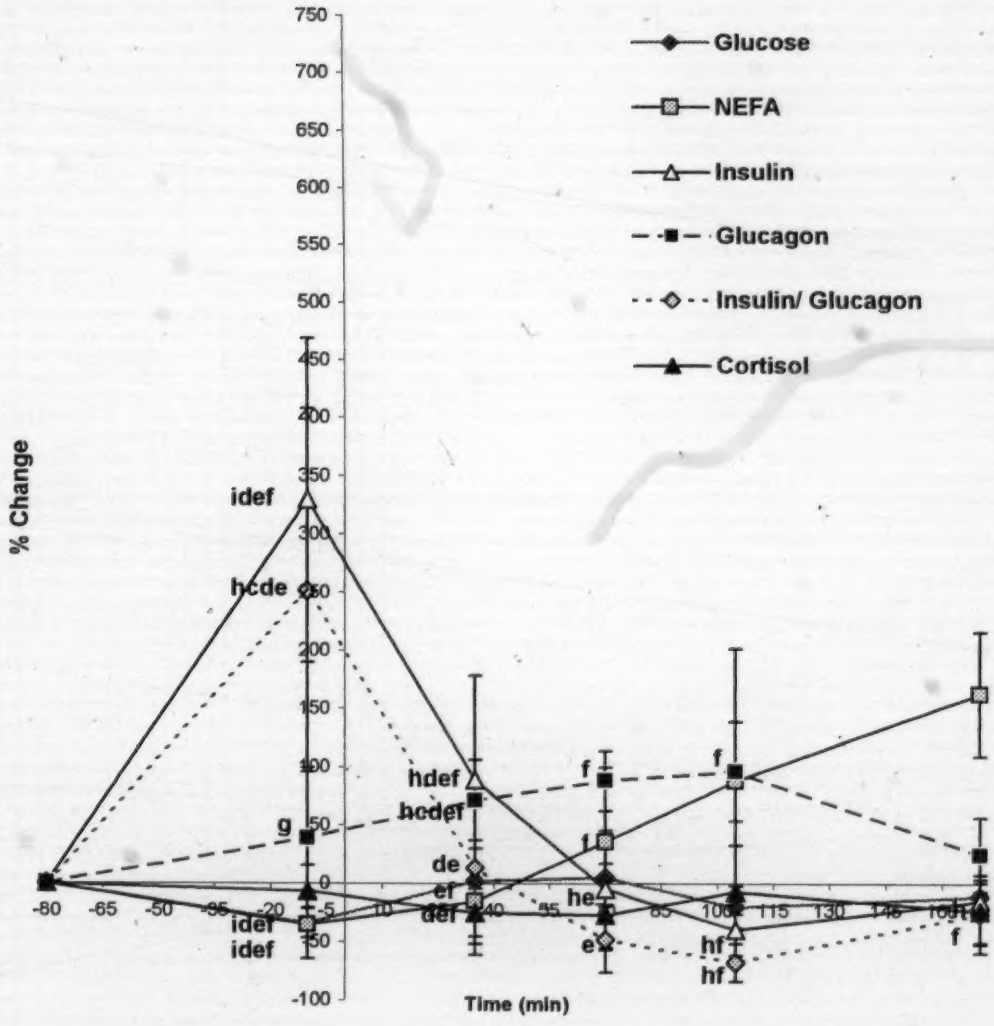


Figure A.9.4: Percent change in blood variables throughout the course of LGI/ LGL (1g CHO/ kg). Values are means ± S.D. **c** = significantly different from 35 min; d = significantly different from 70 min; e = significantly different from 105 min; fsignificantly different from 170 min. h = significantly different from MGI/ MGL trial; i = significantly different from MGI/ HGL trial. $P < 0.05$.

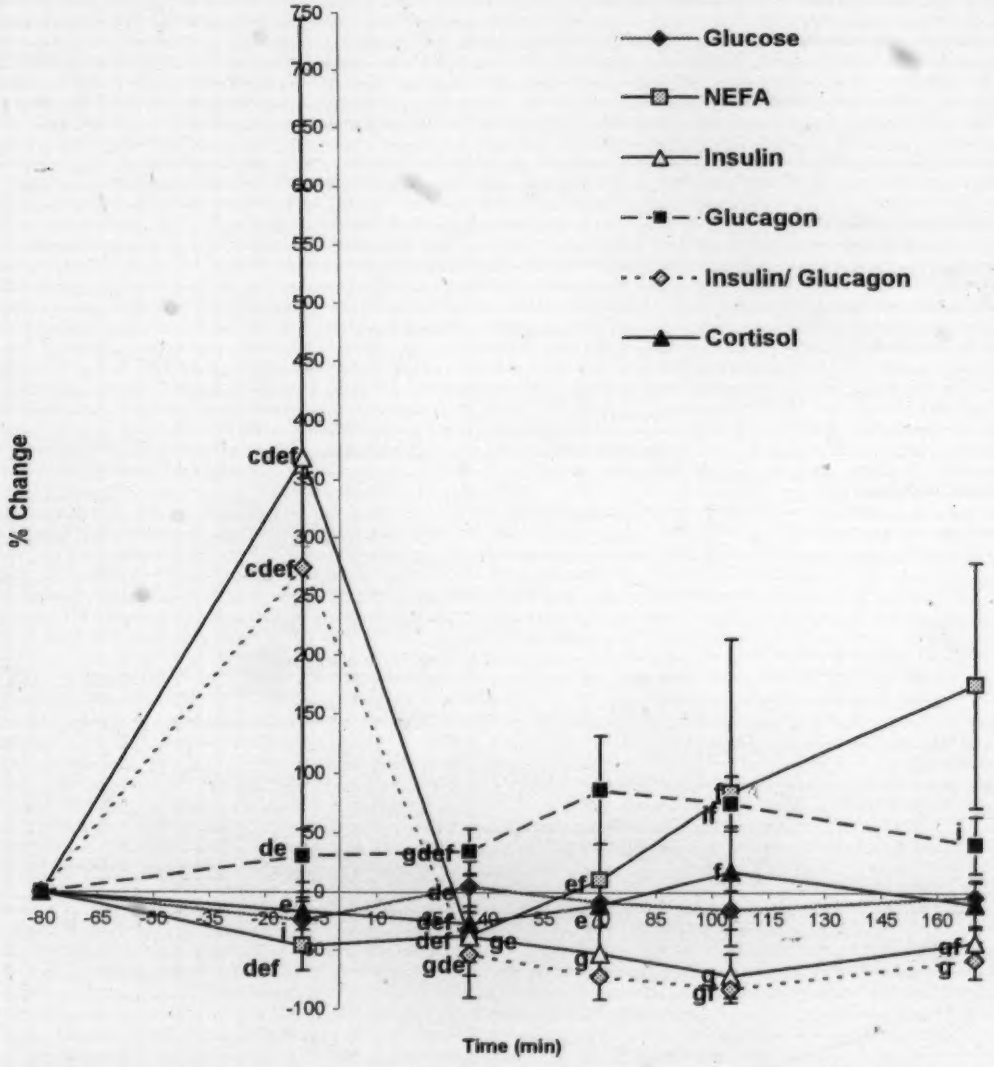


Figure A.9.5: Percent change in blood variables throughout the course of MGI/ MGL (1g CHO/ kg). Values are means ± S.D. c = significantly different from 35 min; d = significantly different from 70 min; e = significantly different from 105 min; f significantly different from 170 min. g = significantly different (lower) from LGI/ LGL trial; i = significantly different from MGI/ HGL trial. $P < 0.05$.

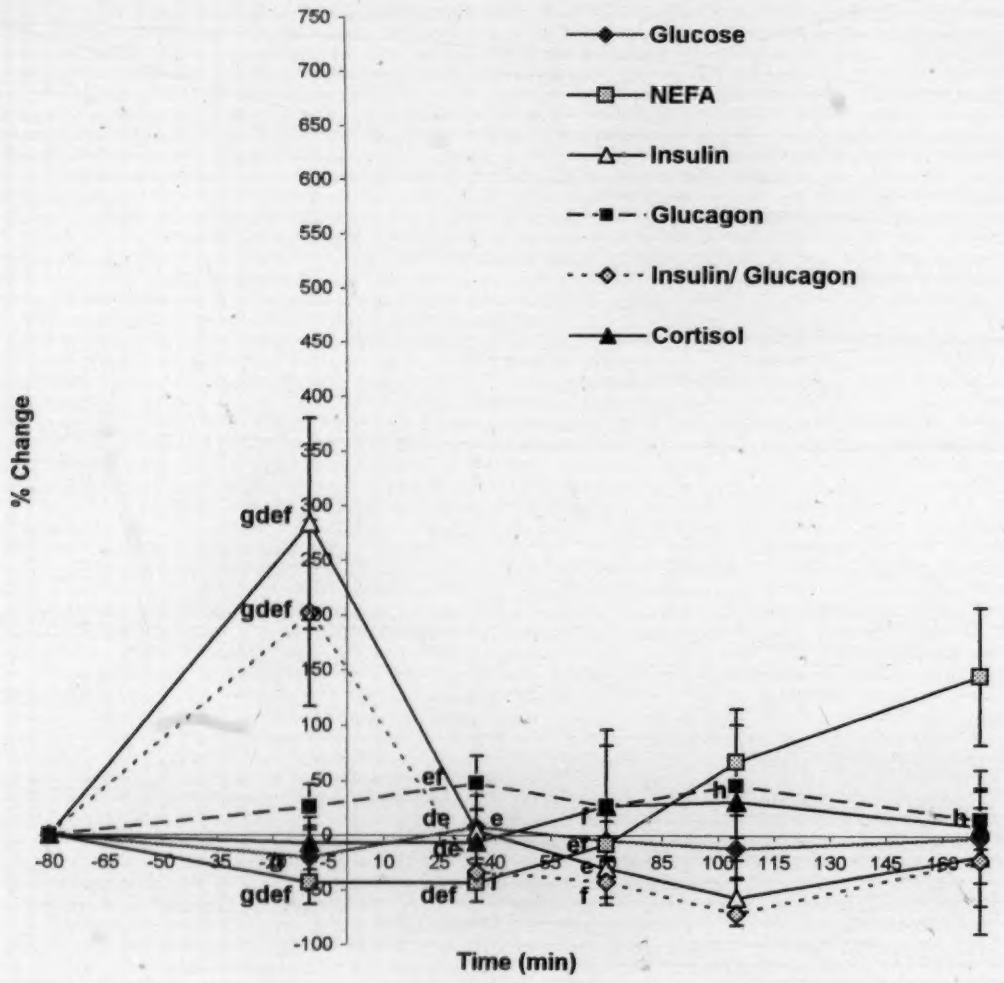


Figure A.9.6: Percent change in blood variables throughout the course of MGI/ HGL (isocaloric). Values are means ± S.D. c = significantly different from 35 min; d = significantly different from 70 min; e = significantly different from 105 min; f significantly different from 170 min. g = significantly different from LGI/ LGL; h = significantly different from MGI/ HGL. $P < 0.05$.

Appendix 10: Assay procedures

NEFA (Non-esterified Fatty Acids): General Principle: NEFA in serum are treated with acyl CoA synthetase (ACS). In the presence of adenosine triphosphate (ATP), magnesium cations and CoA, form the thiol esters of CoA known as acyl-CoA as well as the by products of adenosine monophosphate (AMP) and pyrophosphate (PPI). The further addition of acyl-CoA oxidase (ACOD) results in the oxidation of acyl-CoA and the production of hydrogen peroxide. Hydrogen peroxide in the presence of added peroxidase (POD) allows the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxy-ethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple coloured product. Measuring the optical density of the purple product at its maximum absorption of 550 nm enables the determination of serum NEFA concentrations mathematically using the slope of a standard curve of know concentrations of NEFA.

Glucose Assay General Principle: Glucose oxidase is added to an unknown sample which results in the oxidation of any serum glucose to gluconic acids and hydrogen peroxide. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to form a coloured product, while oxidized o-dianisidine reacts with sulphuric acid to form a more stable coloured product. Measured at 540 nm, the intensity of the pink colour is proportional to the original glucose concentration (Sigma-Aldrich, 2004). Glucose concentration can be determined mathematically using the slope of a standard curve of known concentration of glucose.

Radioimmunoassay General Procedure: In radioimmunoassay a fixed concentration of a radioactively labelled substance is incubated with a constant dilution of antiserum, limiting the concentration of antigen binding sites on the antibody. The addition of an unlabeled antigen results in competitive binding with the labelled tracer for the limited, constant sites on the antibody. The amount of tracer bound to the antibody will consequently decrease as the concentration of the unlabelled antigen increases and visa versa. The amount of unbound tracer can subsequently be measured after separation from antibody bound tracer using a gamma counter to quantify the remaining level of radioactivity. A standard curve can then be constructed with increasing concentrations of standard unlabeled antigen (standards) enabling the determination of antigen amounts from unknown samples (unknowns) using the shape of the curve.

Insulin, glucagon and cortisol determination will all follow the same general principles. Procedures will however differ based on the radioactively labelled tracer used, quantity of unknown required, length of incubation etc. The RIA for insulin for example involves the competitive binding of radioactively labelled I^{125} for a fixed incubation time with the sample serum insulin for sites on the insulin antibody. Following incubation the supernatant is decanted terminating the competition between labelled and unlabelled tracer, whilst isolating the antibody bound antigens which are immobilized on the wall of a polypropylene tube. Use of a gamma counter enables the remaining level of radioactivity to be determined, with the resulting measure used to calculate the concentration of insulin present in the initial sample using the previously constructed standard curve (Diagnostic Procuts Corporation, Los Angeles, CA).